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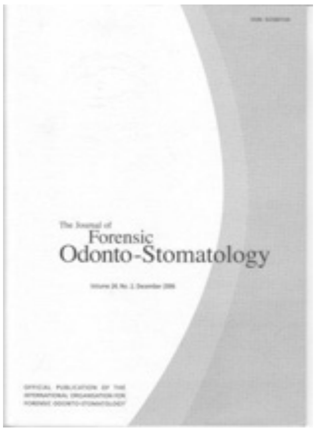

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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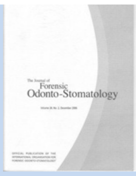

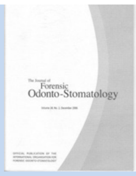

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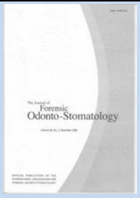

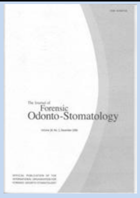

JOURNAL of FORENSIC ODONTO-STOMATOLOGY

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SECTION AGE ESTIMATION

A Comparative Study of Efficacy of Single Rooted and Double Rooted Teeth in Age Estimation Using Dentin Translucency

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ABSTRACT

BACKGROUND: Among various methods of age estimation using dental tissues, measurement of root dentin translucency (RDT) is said to be the most accurate. Numerous studies have estimated age by measuring RDT in single and double rooted teeth and have shown conflicting results. Only few studies have compared efficacy of using single and double rooted teeth for RDT measurement in age estimation.

AIM: To analyze the efficacy of single rooted teeth (SRT) and double rooted teeth (DRT) for measurement of sclerotic dentin (SD) and age estimation.

METHOD: Study was conducted on 120 freshly extracted SR and DR teeth with 60 teeth in each group. Ground sections of 150 µ thickness were observed under stereomicroscope and photographed. The sclerotic dentin length was measured on the images captured using image analysis software.

RESULTS AND CONCLUSION: The present study did not reveal significant difference (p = 0.012) between SRT and DRT when RDT was used for age estimation. However DRT provided more accurate age estimation than SRT with less mean age difference.

KEYWORDS: translucent dentin, single rooted teeth, double rooted teeth, age estimation.

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INTRODUCTION

Age estimation has prime importance in reconstructive human identification especially when there is a lack of ante-mortem data. Dental identification assumes a key role when the deceased person is skeletonized, decomposed, burned, or dismembered". Unlike soft tissues, teeth are extremely resilient and are often well preserved after death without significant loss of micro-structure even after being subjected to temperatures as high as 1600°C.[1]

Various age estimation techniques have been proposed using different skeletal and dental tissues. These methods are either based on the well-ordered cascade of changes that occur during the formation and eruption of teeth or rely on continuous processes that alter the nature of the dental tissues even when individual growth is completed.[2]

Dentine is one of the dental hard substances that is continuously synthesized, maintained and repaired throughout life. Dentine is considered to be an ideal tissue for the study of aging process.[2]

Many methods of dental age estimation have been suggested using parameters such as cementum thickness, dental colour, tooth attrition, secondary dentine formation, and apical resorption. Root dentine translucency has been considered as one of the methods providing accurate results.[3]

Although many studies have been conducted regarding age estimation using root dentine translucency, conflicting results are presented in respect of the selection of teeth used in the studies. Some studies have suggested that the measurement of root dentine translucency in single-rooted teeth is the only technique to give accurate results[2,4]. Other studies have demonstrated that double-rooted teeth showed significant correlation with the known age in relation to the determined age compared to results obtained when single-rooted teeth were used.[5]

Therefore, the aim of the present study was to compare the accuracy of age estimation by the measurement of root dentine translucency using both double and single-rooted tooth.

MATERIALS AND METHODS

120 freshly extracted teeth with an equal number of single-rooted teeth (SRT) and double-rooted teeth (DRT) were collected from individuals between 20 -50 years of age. Following explanation of the study design, written consent and a detailed case history was recorded from each individual. Institutional ethical clearance was requested and granted.

Single-rooted and double-rooted teeth (maxillary first premolars, mandibular molars) extracted for therapeutic reasons were included in the study. Endodontically treated teeth, teeth with history of trauma, teeth associated with cysts and tumors and impacted and non-vital teeth were excluded. All the teeth were fully erupted.

Each study group was further subdivided depending on age as 21-30 years (S1 & D1), 31-40 years (S2 & D2), and 41-50 years (S3 & D3) in which S denotes single rooted and D denotes double rooted teeth.

Immediately after extraction, the teeth were thoroughly rinsed in normal saline solution and preserved in 10% neutral buffered formalin until ground sections were prepared. Each dried tooth was embedded in autopolymerizing acrylic resin and longitudinal sections of 150 µ thickness in the axio-bucco-lingual plane were obtained using a hard-tissue microtome (LEICA. SP1600, Germany) (Fig 1). The ground sections were mounted on a glass slide using DPX mounting media and a coverslip was placed over them. All slides were coded to ensure blinding and viewed using a stereomicroscope (Olympus SZx 12 Japan). The photomicrographs of ground sections of teeth were captured using stereomicroscope attached to 3 chip CCD camera (Proview, media Cybernetics USA) (0.5 x magnifications) under 5x magnification objective and stored in the computer (17" monitor and Intel Pentium III processor windows 95/ NT/ 98, media cybernetics, USA) for further processing. Following calibration the length of the apical translucent dentine was measured in millimetres using image analysis software (Image Proplus version 4.1, media cybernetics, USA. The measurements were stored in Microsoft Excel for further statistical analysis.

In single-rooted tooth, the root dentine translucency was measured on the side of the root that showed the greatest translucency length apico-coronally (Fig 1). In double-rooted tooth, the root dentine translucency was measured from both the roots and mean of these measurements was calculated (Fig 2).

The modified Bang & Ramm's formula[6] for an Indian population is detailed below. By input of the measurements for each individual their age was estimated.

- Linear regression (if $T \geq 9$mm): $Age = 35.5619 + (3.4828 \times T)$
- Quadratic regression (if $T \leq 9$mm): $Age = 29.9074 + (7.4507 \times T) + (-0.4369 \times T^2)$

Where, T = Length of apical translucent dentin.

All of the data obtained were recorded in tabulated format and subject to statistical analysis using regression analysis and unpaired t-test for comparison with the study group. Student unpaired 't' test was used for the inter-group comparison and Tukey post-hoc test was used for the pair wise intra-group comparisons. Repeat measurements of the length of root dentine translucency were recorded by two observers and the measurements were subjected to paired t-test to assess potential intra- and inter-observer error.

RESULTS

The mean and standard error of estimated age using root dentine translucency in single-rooted and double-rooted teeth was 7.53±2.4 and 6.05±1.4 respectively. The mean age difference on inter-group comparison was statistically insignificant ($p = 0.012$). However, double-rooted tooth showed less mean age difference when compared to single-rooted teeth (Table 3).

The mean age difference between known age and estimated age showed a gradual increase with advancing age in both the study groups (Table 1 and 2).

Intra-group comparison in single-rooted teeth revealed statistically insignificant results similar to double-rooted teeth. However there was an exception between D3 and D1 where a statistically significant difference was observed ($p=0.004$) (Table 1 and 2).

Age wise intra-group comparison of single-rooted and double-rooted teeth revealed statistically insignificant results. However there was an exception between S1 and D1 where a statistically significant difference was observed ($p=0.003$) (Table 4).

Correlation analysis of single and double-rooted teeth was positive with a percentage correlation of 85% and 88% respectively and a standard error of 2.4 yrs and 1.4 yrs respectively (Table 5). A positive correlation was also found between root dentine translucency and advancing age (Table 6).

Inter-observer variability (Table 7) and intra-observer variability (Table 8) regarding the measurement of root dentine translucency in single and double-rooted teeth revealed there was no statistically significant difference.

Table 1 - Intra-group measurement and comparison of translucent dentin length in single rooted teeth

Single rooted teeth	Age groups (in years)	Mean value of transparent dentine in length (mm)	Difference between chronological age and estimated age (M±SE)	P value of ANOVA	Post Hoc Tukey test (Intra-group comparison)
S1	21-30	0.15	6.55±0.344	0.208 (NS)	S1 and S2 – 0.542(NS)
S2	31-40	2.04	7.65±0.822		S2 and S3 – 0.751(NS)
S3	41-50	4.66	8.40±0.913		S3 and S1 – 0.184(NS)

NS- Not Significant, mm-millimeter, M- Mean, SE- Standard Error.

Table 2 - Intra-group measurement and comparison of translucent dentin length in double rooted teeth

Double rooted teeth	Age groups (in yrs)	Mean value of transparent dentine in length (mm)	Difference between chronological age and estimated age (M±SE)	P value of ANOVA	Post Hoc Tukey test (Intra-group comparison)
D1	21-30	0.05	4.50±0.550	0.006(NS)	D1 and D2 - 0.144(NS)
D2	31-40	2.06	6.20±0.894		D2 and D3 – 0.244(NS)
D3	41-50	4.45	7.45±0.366		D3 and D1 – 0.004(S)*

S*- Significant, NS- Not Significant, mm-millimeter , M- Mean, SE- Standard Error

Table 3 - Intergroup comparison of translucent dentin length between single rooted and double rooted teeth

Study groups	Estimated age (M±SE)	Mean value of transparent dentine in length (mm)	Un-paired t test
Group 1- Single rooted teeth	7.55±2.4	2.37	0.012(NS)
Group 2 - Double rooted teeth	6.02±1.4	2.35	

NS- Not Significant, M- Mean, SE- Standard Error, mm-millimeter

Table 4 - Age wise intergroup measurement and comparison of translucent dentin length between single and double rooted teeth

Groups 1 versus Group 2	Age groups (years)	Un paired t test
S1 vs D1	21-30 yrs	0.003(S)*
S2 vs D2	31-40 yrs	0.229(NS)
S3 vs D3	41-50 yrs	0.340(NS)

S*- Significant, NS- Not Significant

Table 5 - Correlation between chronological age and estimated age of single and double rooted teeth

		r	p	r²	SE
Correlation between	Chronological Age and Estimated age using single rooted teeth	0.92	<0.000	85%	2.4 yrs
	Chronological Age and Estimated age using double rooted teeth	0.94	<0.000	88%	1.4 yrs

$p < 0.000$ HS, r – Correlation coefficient, r^2 – Percentage correlation, SE- Standard error

Table 6 - Correlation between root dentine translucency with increasing age

Correlation of dentin translucency with increasing age	r	p	r²	SE
	0.91	0.000	83%	0.015

$p < 0.000$ HS, r – Correlation coefficient, r^2 – Percentage correlation, SE- Standard error

Table 7 - Inter-observer variation in measuring root dentine translucency length among single and double rooted teeth

Inter-observer variability	Study groups	Number of teeth	p-value	R
	Single rooted teeth	60	0.437(NS)	0.98
	Double rooted teeth	60	0.999(NS)	0.97

NS: Not Significant, r – Correlation coefficient

Table 8 - Intra-observer variation in measuring root dentine translucency length among single and double rooted teeth

	Study groups	Number of teeth	p-value	R
Intra-observer variability	Single rooted teeth	60	0.254(NS)	0.99
	Double rooted teeth	60	0.765(NS)	0.99

NS: Not Significant, r – Correlation coefficient

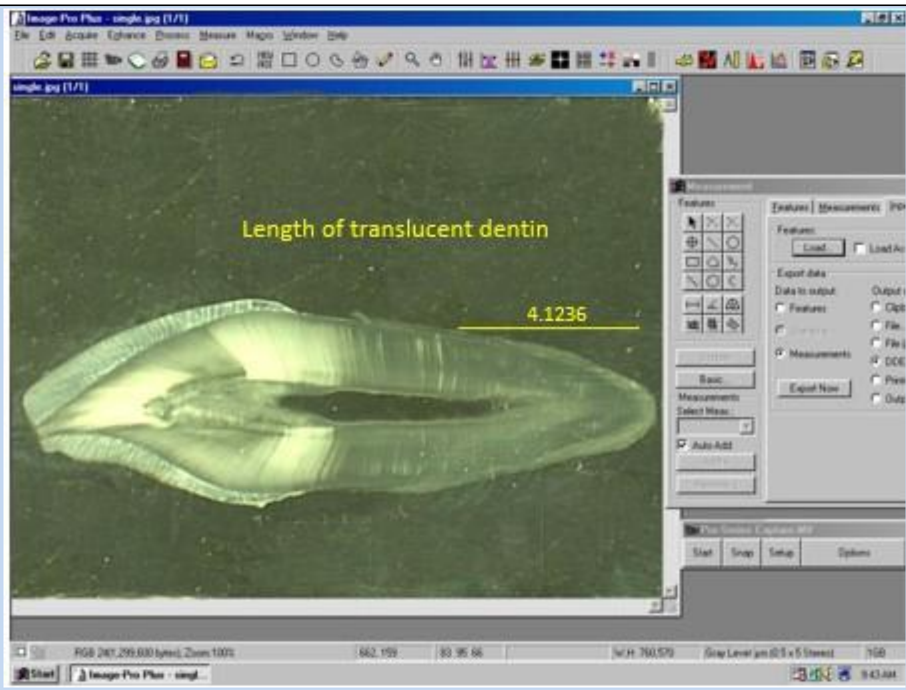

Fig.1: Photomicrograph showing measurement of translucent dentin length in single rooted tooth using image analysis software (5x magnification)

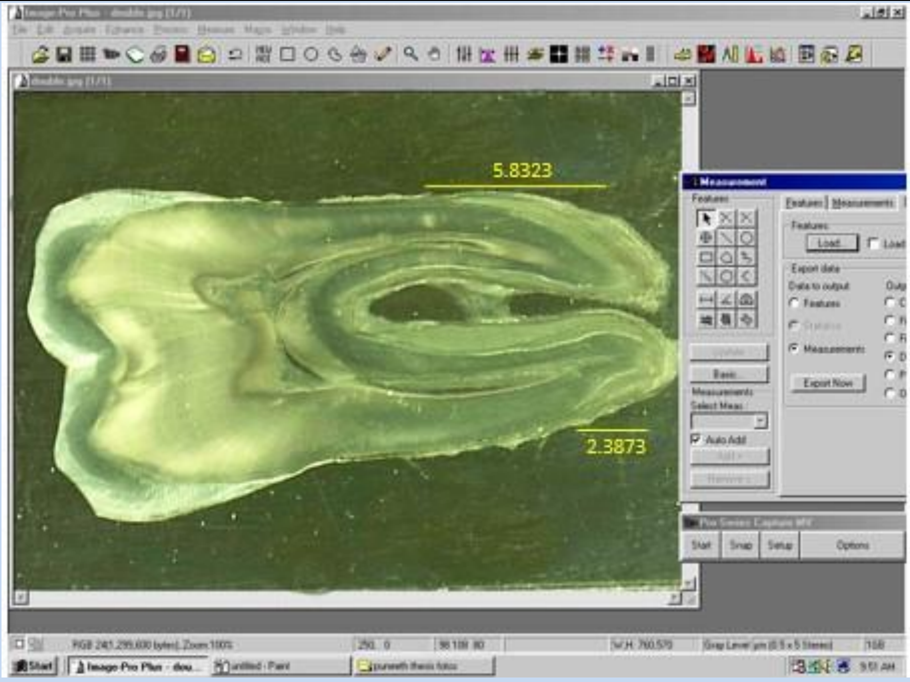

Fig.2: Photomicrograph showing measurement of translucent dentin length in double rooted tooth using image analysis software (5x magnification)

DISCUSSION

Age estimation is of great importance for the identification of unknown victims or skeletal remains in cases where trauma, crime or disaster are a feature of the case.[7] Teeth are the most durable structures in the human body. In many archaeological sites or forensic cases, the teeth are the only human remains.[8]

Gustafson's seminal work sowed the seed for future generations of researchers to investigate the changes that occur in teeth that could be used for purposes of age estimation. Among the six variables suggested by Gustafson, root dentine translucency was found to be the easiest of the six variables to assess. Gustafson (1950) first observed that root dentine becomes transparent with age, with the process beginning at the apex of the root and proceeding towards the crown of the tooth [9]. Root dentine translucency is considered to be least affected by external stimuli and most suitable for the purpose of age estimation.[10]

In the present study, a strong correlation between the length of translucent dentine and advancing years of age was found. A definite and gradual increase in the extent of the root dentine translucency with advancing years of age was noted. Previous studies have demonstrated that the extent of the root dentine translucency can be a reliable indicator of individual's age.[9, 11, 12]

In the present study the mean age difference between chronological and estimated age for the whole sample using the root dentine translucency method was 6.7 years. A value <±10 years is considered as "acceptable" in forensic age estimation, and this mean difference of estimated age in the present study was less than that reported by Acharya et al (8.3 yrs), [6] Bang and Ramm et al (11.2 yrs),[11] Meinl A et al (9.9 yrs),[2] Singhal et al (15.6 yrs),[13] and Lamendin et al (8.4yrs).[4]

It should be noted that there are some studies where the reported a mean age difference is less than that reported in the present study with mean difference of 3.5-6.5 yrs.[14, 15]

Observer variability:

Measurements were carried on 60 sections of single and 60 sections of double-rooted teeth by two observers. Inter-observer variability showed no statistical significant difference in both single-rooted (p= 0.437) and double-rooted teeth (p= 0.999) with Pearson's correlation coefficient of 0.98 and 0.97 respectively (Table 7). Intra-observer variability did not reveal statistical significant differences in both single-rooted (p= 0.254) and double-rooted teeth (p= 0.765) with Pearson's correlation coefficient of 0.99 and 0.99 respectively. The insignificant variations observed between the examiners indicated that measurement of root dentine translucency produces consistent results when undertaken by different examiners. These results were in accordance with the study carried out by Ashith and Vimi.[6]

Age estimation using root dentine translucency in single rooted teeth:

The mean age difference of 6.55, 7.65 and 8.40 between the estimated age and chronological age in single-rooted teeth was not statistically significant (p= 0.574) in the age groups of 21-30 yrs, 31-40 yrs and 41-50 yrs suggesting that value considered is acceptable for age estimation in all of these age groups.

Two previous studies showed a greater mean age difference between estimated and chronological age compared to the present study. Meinl A et al[2] showed a mean difference ranging between 10.5-17.3 yrs for the three age groups (10.5, 12.3, 17.3 yrs) and Lamendin H et al.[4] showed a mean age difference ranging between 3.3-13.3 years in four study groups (30-39, 40-49, 50-59, 60-69 yrs). This difference may be explained by the use of population specific formulae in this study which was specific to an Indian population.

When intra-group comparison of the mean age difference between chronological age and estimated age was carried out in single-rooted teeth the difference between the age of S1 and S2, S2 and S3 and S3 and S1 groups was not statistically significant (p= 0.542, p= 0.751, p= 0.184 respectively)

Formation of translucent root dentine usually begins in the 3rd decade (21-30 yrs),[2, 11] and this feature was also observed in five samples of the present study. Few studies have reported the formation of translucent root dentine below 20 years of age and the reason for the formation of translucent root dentine translucency in this age group is still unexplained.[3, 16] It should be noted that in our study no subjects were below 20 years of age.

In the present study, a strong correlation of 0.92 was observed between estimated age and actual age of single rooted teeth similar to other studies with different values (r=0.80, r=0.81 and r=0.78 respectively).[5, 11, 13]

Age estimation using root dentine translucency in double rooted teeth:

The rationale for selecting double-rooted teeth was that the measurement of more than one root would produce more accurate results. Three-rooted teeth were not included in the study because of difficulty in sectioning three-rooted teeth and also because of the limited availability of three-rooted teeth.

The mean age difference of 4.50, 6.20 and 7.45 between the estimated age and chronological age in double-rooted teeth was not statistically significant (p= 0.006) in age groups 21-30 yrs, 31-40 yrs and 41-50 yrs respectively suggesting that the values could be acceptable for age estimation in these age groups. These values could not be compared to any other studies since no study has been carried out in double rooted teeth in different age groups.

When intra-group comparison of mean age difference was carried out in double-rooted teeth, the mean age difference between the D1 and D2 and D2 and D3 groups was not statistically significant ($p= 0.144$, $p= 0.244$ respectively), whereas the mean age difference between D3 and D1 was statistically significant ($p= 0.004$). This difference could be as a result of more subjects in group D3 who were more than 45 years of age.

In the present study, a strong correlation of 0.94 was obtained between estimated and actual age of double-rooted age, which was similar to study done by Brkic H et al.[5]

Inter-group comparison of mean age difference between single and double-rooted teeth:

In the present study comparison of the overall mean age difference of single-rooted teeth with that of double-rooted teeth demonstrated that there was no statistically significant difference ($p=0.012$) with a mean and standard error 7.53±2.4, 6.05±1.4 respectively. This implies that both types of teeth i.e. single and double-rooted teeth can be used for purposes of age estimation. However double-rooted teeth showed less mean age difference when compared with single-rooted teeth.

Age wise inter-group comparison of the mean age difference between single-rooted teeth and double-rooted teeth demonstrated a statistically significant difference between S1 and D1 (21-30 yrs) ($p=0.003$). An explanation for this difference could be that teeth in this age group showed minimal or no formation of root dentine translucency. Additionally this difference could be attributed to the age distribution of subjects in the groups S1 and D1. Only 4 subjects in group S1 were more than 25 years of age while in group D1 9 subjects were over 25 years of age. It should be noted that the differences between the other groups S2 and D2 (31-40 yrs) and groups S3 and D3 (41-50 yrs) were not statistically significant ($p= 0.229$, $p= 0.340$ respectively).

The correlation coefficients of estimated age with the actual age using root dentine translucency of single and double-rooted teeth was 0.92 and 0.94 respectively, indicating that both type of teeth can be used for estimating age.

Previous studies have reported a variety of correlation coefficients for the extent of root dentin translucency with age; $r= 0.73$ (Miles, 1983); $r= 0.65$-0.83 (Bang and Ramm, 1970); $r= 0.86$ (Johanson, 1971); $r=0.75$ (Azaz, 1977); $r=0.87$ (Vasiliadis et al., 1983)} [16].

The results of the present study also fall within these previously published figures i.e. $r= 0.83$, with the exception of Chinese and Malays where root dentine translucency did not correlate highly with age.[16]

FUTURE SCOPE

Although the formation of root translucent dentine is said to be an age-related change, few studies have reported root translucent dentine to be present in subjects below 20 years of age. The reason for this anomaly is not explained. For this reason the present study did not include subjects below 20 years of age. Further studies including subjects from both the age groups 11-20 yrs and 21-30 yrs should provide an insight into the process involved in the formation of root translucent dentine. The formation of translucent root dentine is also thought to be affected by other factors including sex, type of tooth and dental arch. These variables could also be included in future studies.

CONCLUSION

Both single-rooted and double-rooted teeth showed high correlation for purposes of age estimation. However double-rooted teeth showed less mean age difference and standard error when compared with single-rooted teeth suggesting that double-rooted teeth provide a more accurate method of age estimation than single-rooted teeth. The minimum age that can be calculated using the modified Bang and Ramm formula is 29.9 years and this precludes any application for use with younger age groups. Use of the modified Bang and Ramm formula in studying samples obtained for subjects less than 30 years is inappropriate and this fact should be considered in the future studies where translucent root dentine is used for purposes of age estimation.

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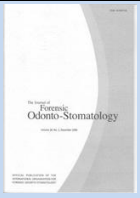

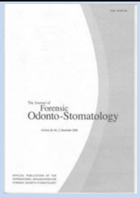

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SECTION AGE ESTIMATION

Assessing age-related change in Japanese mental foramen opening direction using multidetector computed tomography

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The authors declare that they have no conflict of interest.

ABSTRACT

***Objective:** The purpose of this study was to investigate how the opening direction of the mental foramen (MF) changes with age in a Japanese population using multi-detector computed tomography (MDCT). **Methods:** Post-mortem MDCT scans of 121 Japanese subjects (66 males and 55 females) were carried out where all subjects possessed at least twenty teeth, including molar teeth, in the upper and lower jaws. Two angles of the mental foramen opening were measured, namely the superior-inferior angle in the coronal plane and anterior-posterior angle in the transverse plane, on the CT reconstructed images. The associations between age and these two angles were evaluated using a multiple regression analysis. **Results:** For male subjects, the relationship between the superior-inferior angle and age was a quadratic curve (p<0.001). This angle increased until the subject reached their early 50s and then the angle decreased with age. In the transverse plane, there was a linear relation between the anterior-posterior angle and age (p=0.002).It was noted also that the angle decreased with age. By contrast, however, no significant associations between the two angles and age for either measurement were noted for female subjects. This study demonstrated that the opening direction of the mental foramen changes with age in Japanese male subjects. By contrast this change in the opening direction of the mental foramen was not demonstrated in Japanese female subjects. In male subjects, the opening direction moves superiorly until the individual reaches their early 50s, and then moves inferiorly with advancing age. It also shifts from a posterior to an anterior position with age. **Conclusion:** These observed change differ from the results of previous studies. The findings could be useful for forensic science as they demonstrate a change in the position of mental foramen in a sample of contemporaneous male Japanese subjects.*

KEYWORDS: forensic odontology, forensic anthropology, postmortem computed tomography imaging, aging, Japanese

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INTRODUCTION

The mental foramen (MF) is located under the mandibular second premolar tooth and opens postero-superiorly. It is an exit of the mandibular canal, and the inferior alveolar nerve and blood vessels pass through it. Once they exit the MF, the nerve and vessels become MF vessels and nerves and conduct the distribution of the sensory nerve and vascular supply to the soft tissues of the chin, lower lip, and the posterior gingiva of the second mandibular molar region.[1,2] For this reason, the MF is an important landmark in clinical dentistry for the purposes of nerve block and dental implant or oral surgery.

In the forensic field, the MF is one of the anatomical landmarks to assist the identification of human remains, and various forensic anthropological studies of MF shape, size and position have been conducted. Amorim et al. and Apinhasmit et al. reported on the relationship between MF location and sex in dry mandibular bone.[3,4] Cutright et al. described racial trends in MF position by examining skulls.[5] With regard to changes in MF due to aging, Gershenson et al. evaluated the MF location in dry mandibles and reported that MF changed with age.[6] Kamijyo[7] and Takenoshita[8] measured Japanese dry mandibular bone to examine age-related changes of MF by performing macroscopic observations.

Computed tomography (CT) is a suitable modality for observing hard tissue and has been widely used in the field of medicine, including clinical dentistry (e.g., dental implants and maxillofacial surgery). It has also been used to evaluate the mandibular canal after (??) the MF.[9] The use of CT is also becoming more common in the practice and study of forensic medicine.[10-13] Postmortem CT images taken in a forensic field are also applied to some anthropological studies.[11] However, no studies have focused on the change of the MF with age using MDCT. In the present study the opening direction of MF in Japanese subjects was evaluated using multi-detector raw computed tomography (MDCT) and assessed whether it changed with age.

MATERIALS AND METHODS

Cadavers scheduled to undergo forensic autopsies were examined between January 2010 and February 2014 at the department of Legal Medicine of Chiba University. The cadavers used in this study were selected from Japanese subjects who had at least 20 remaining teeth including molar teeth in the upper and lower jaws. Edentulous cases and cases where the loss of [posterior teeth had led to occlusal collapse were excluded. The cadavers with mandibular asymmetries and fractures were also excluded. In total, MDCT was used to measure the angles of the opening direction of the MF in 121 cadavers (66 males and 55 females). The present study gained the approval of the ethics committee of Chiba University. The sex and age distributions are provided in Table 1.

Prior to forensic autopsy, postmortem MDCT scans (16-section MDCT scanner, Eclos, Hitachi Ltd, Tokyo, Japan) were carried out using the following protocol; 0.625 mm collimation, 0.63mm reconstruction interval, 120 kV tube voltage, 200 mA tube current, and 1 r/s rotation time. Image processing was performed on a radiological work-station (SYNAPSE VINCENT, Fujifilm, Tokyo, Japan). Firstly the mandibular plane was defined as a three-dimensional (3D) image on the work-station denoted by tangent lines drawn from the nadir of the mental region in the median sagittal plane (Me) to the right and left mandibular inferior margins (Fig. 1). The perpendicular and parallel planes were the reconstructed on the basis of this mandibular plane then the "coronal" and "transverse" planes were defined to observe and measure the MF. The coronal plane was defined as the plane that best describes the MF maximal opening of these perpendicular planes to the mandibular plane as shown in Fig. 2-A. The transverse plane was defined as the plane that best describes the MF maximal opening of these parallel planes to the mandibular plane as shown in Fig. 3-A. On each plane two parameters were measured of the MF opening direction as described below:

I. Opening direction of the MF in the coronal plane (Angle θ1, Fig. 2)

The superior-inferior angle was measured, indicating the MF opening direction based on the definition of Sasaki et al[9] (Fig. 2-A, B). On the coronal plane, two lines were drawn. One was Line a passing through each midpoint of the MF opening on the outside and inside of the cortical bone (Fig. 2-A, B), and the other was Line b parallel to the mandibular plane (Fig. 2-A). The angle consisting of these two lines was Angleθ1.

Table 1: Sex and age distribution

	10's	20's	30's	40's	50's	60's	70's	over80's	Total
Males	8	9	10	10	10	9	8	2	66
Females	2	8	4	11	11	8	8	3	55

Fig.1: The mandibular plane consists of the most inferior point of the chin and both mandibular inferior border.

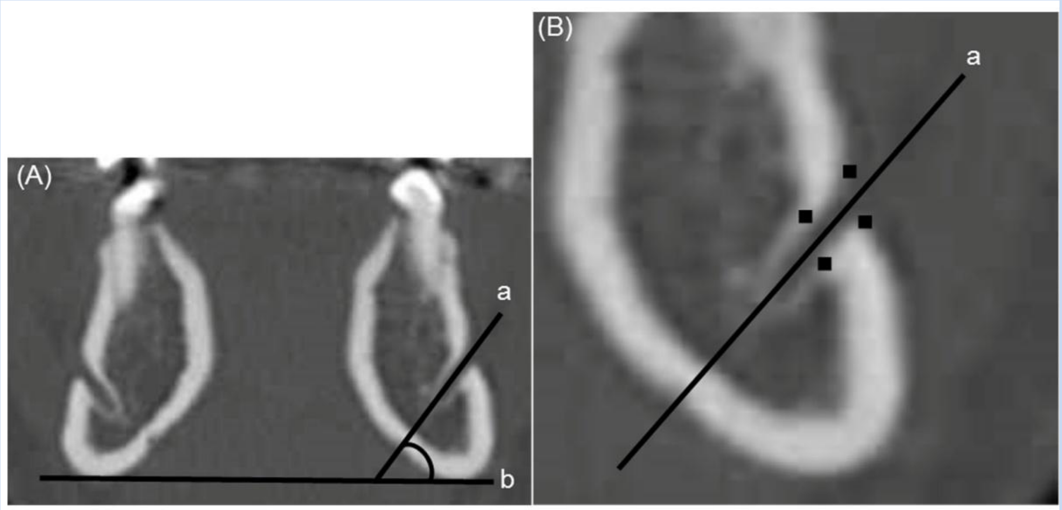

Fig. 2: Opening direction of the mental foramen (MF) in the coronal plane A. the superior-inferior angle in the coronal plane consists of Line a passing through the middle of the MF and Line b parallel to the mandibular plane B. the enlarged figure of the MF maximal opening.

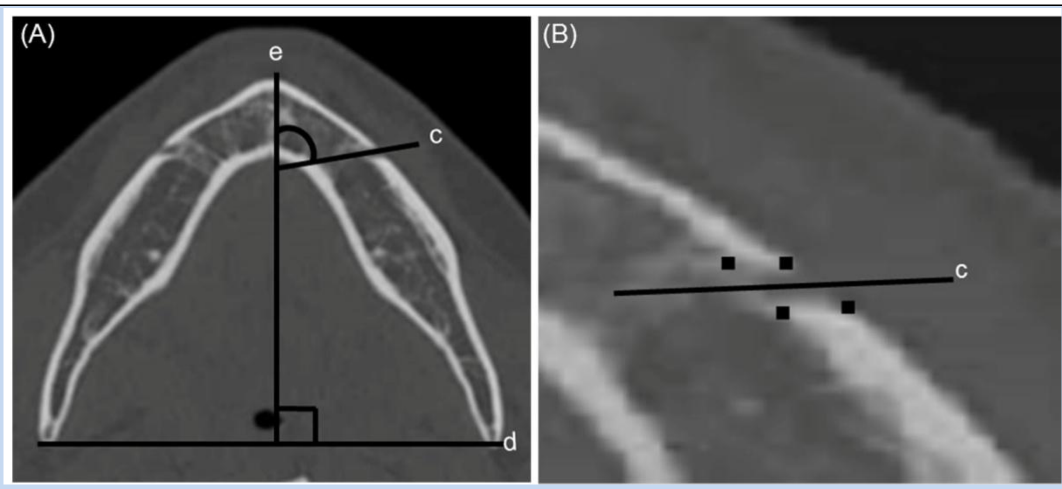

Fig. 3: Opening direction of the mental foramen (MF) in the transverse plane A. the anterior-posterior angle in the transverse plane consists of Line c passing through the middle of the MF and Line e perpendicular to Line d which links bilateral posterior margins of mandibular ramus. B. the enlarged figure of the MF maximal opening.

II. Opening direction of MF in the transverse plane (Angle θ2, Fig. 3)

The anterior-posterior angle was measured indicating the opening direction of the MF (Fig. 3-A, B). On the transverse plane, three lines were drawn. One was Line c passing through each midpoint of the MF opening on the outside and inside of the cortical bone (Fig. 3-A, B), another was Line d linking the right and left posterior margins of the mandibular ramus (Fig. 3-A), and the other was Line e perpendicular to line d (Fig. 3-A) . The angle consisting of Lines c and e was Angle θ2. Measurements of Angle θ1 and Angle θ2 were performed on each MF maximal opening, and by sex. In each measurement, measurements on both the left and right sides of the MF were taken, and the average of these two measurements was calculated. Two weeks later 20 MDCT images were randomly selected and MF was measured once again. The relative technical error of measurement (rTEM, %) and the coefficient of reliability (R) were calculated for estimation of the intra-observer error.[14-16] It was considered that rTEM (%) < 5% and R value > 0.75 was sufficiently precise.[15,16]

The associations between age and MF opening directions (Angles θ1 and θ2) were evaluated using multiple regression analyses. To confirm quadratic relationships age was entered into the model using a forced entry method and age square term using a forward elimination method with a threshold of $p < 0.05$. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics software version 20.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Using post-mortem MDCT images of 121 Japanese cadavers, age-related changes in the opening direction of the MF in the coronal plane (Angle θ1) and transverse plane (Angle θ2) were investigated. The intra-observer measurement error of Angle θ1 was expressed as rTEM =2.60% and R=0.99 (95% confidence interval 0.966~0.994). In Angle θ2, it was rTEM = 4.20% and R =0.97 (95% confidence interval 0.919~0.987). These results were within accepted standards.

In females, no significant association was demonstrated ($p = 0.709$) between age and Angle θ1 (Table 2). No significant association was demonstrated ($p = 0.392$) between age and Angle θ2 (Table 3).

However, when the association of age and the opening direction of MF in the coronal plane of male subjects was examined, the age-squared term was significant ($p < 0.001$) for the change of Angle θ1. Notably, the relationship between them was quadratic curvilinear (Fig. 4). The regression equation that explained the change of Angle θ1 with age was as follows: Angle θ1 = 24.06 – 0.01 × (age)² + 1.04 × age, and the coefficient of determination (adjusted R^2) was 0.178 (Table 2). Based on the quadratic curve obtained from the regression equation, Angle θ1 increased until subjects reached their early 50s and thereafter decreased with age.

In the transverse plane, the age was significant ($p = 0.002$) for the change of Angle θ2. The relationship between age and the opening direction of the MF (Angle θ2) of males exhibited a linear relationship (Fig. 5). The regression equation of age-related change of Angle θ2 was as follows: Angle θ2 = 86.57 – 0.26 × age, and adjusted R^2 was 0.131 (Table 3). This regression equation demonstrated that Angle θ2 decreased with age.

DISCUSSION

The purpose of the present study was to assess how the opening direction of the MF changed with age in a dentate Japanese population sample with no occlusal abnormalities as a result of the loss of posterior molar teeth using MDCT. The present study demonstrated age-related changes in MF opening directions in male subjects. By contrast no age-related changes were demonstrated in MF opening directions in female subjects.

Unlike the results for male subjects, no significant correlations between age and the opening direction of the MF in the coronal or transverse plane was detected for female subjects. In this present study it is recognized that only a limited number of young female subjects were included in the sample size. It is recognized that the muscles (including those responsible for occlusal force) are weaker in females than in males. Utsuno et al. reported that facial soft thickness differences were greater in females than that in the case of males.[17] It is considered that there is more variation in the individual cranial morphologies of female subjects compared to the individual cranial morphologies of male subjects. This may offer an explanation for the gender-based differences demonstrated in the present study.

In male subjects, the opening direction of the MF moved superiorly part with age until the early 50s, after which time it moved inferiorly. The morphological changes in mandibular bone after middle age due to age-related periodontal disease,[18] may have some influence on the opening direction of the MF. Similarly, the decrease in mandibular mineral content as a result of ageing might also influence this variable.[19, 20]

Table 2: The relationship between age and MF opening direction in the coronal plane

	Regression coefficient (95% confidence interval)	*p*-value	Adjusted R^2
Males			
Age	1.044(0.513, 1.576)	< 0.001	0.178
$(Age)^2$	-0.010(-0.016, -0.005)	< 0.001	
Females			
Age	-0.032(-0.201, 0.137)	0.709	-0.016

Age was entered in the model using a forced entry method and an age squared term was entered using a forward elimination method with a threshold of $p < 0.05$.

Table 3: The relationship between age and MF opening direction in the transverse plane

	Regression coefficient (95% confidence interval)	*p*-value	Adjusted R^2
Males			
Age	-0.262(-0.422, -0.103)	0.002	0.131
Females			
Age	-0.082(-0.273, 0.109)	0.392	-0.005

The opening direction of the MF tended to move anteriorly with age in males, and this finding was different from the results of previous research studies. Al-Khateeb et al. reported that the MF position of subjects in northern Jordan increased with advancing age using panoramic radiographs; specifically, there was a greater frequency of more posterior and inferior positioning.[21] Santini et al. also reported that the MF in mandibles of Chinese and British skulls became more distal with age.[22] In Japanese subjects, Kamijyo[7] and Takenoshita[8] performed mandibular macroscopic observations. Specifically Takenoshita[8] inserted needles into the MF of 40 dry mandibles from Japanese subjects to investigate the MF opening direction. Kamijyo[7] and Takenoshita[8] reported that the MF opening moved from the anterior to posterior direction with age. The use of differing measuring methods is likely to account for discrepancies in their findings. Cutlight et al. reported that the MF of Negroid subjects were posterior compared with those in Caucasoid subjects[5] and that racial differences may be a factor affecting the position of the MF . It is recognized that bone formation can be influenced by dietary habits, environmental influences, and socioeconomic factors.[23]

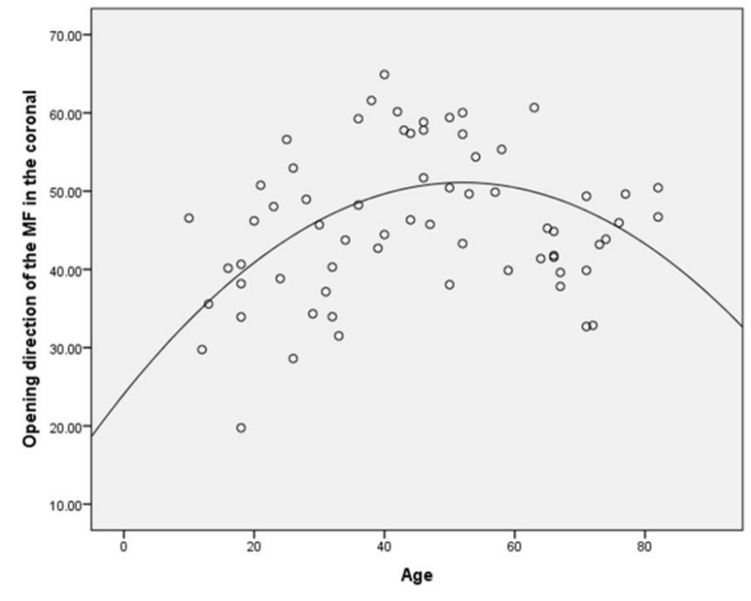

Fig. 4: Age-related change in the mental foramen (MF) opening direction in the coronal plane.

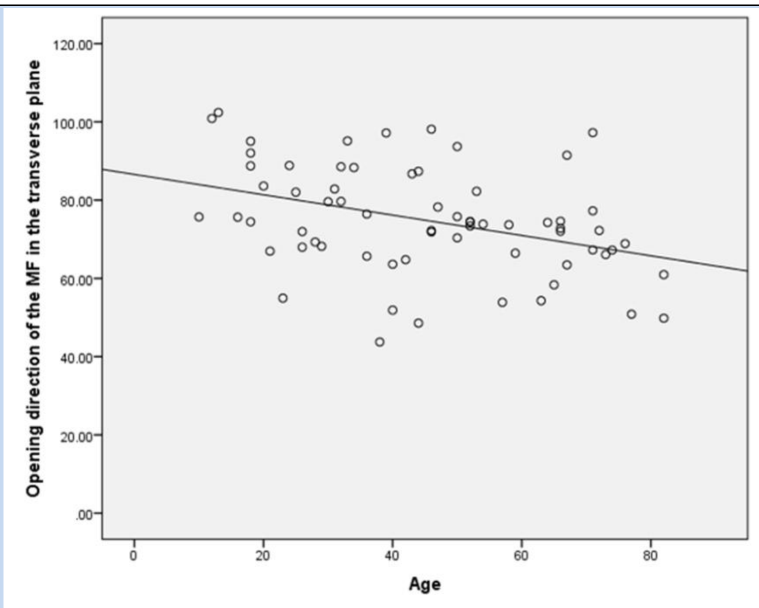

Fig. 5: Age-related change in the mental foramen (MF) opening direction in the transverse plane.

With regard to the findings of Kamijyo[7] and Takenoshita,[8] both reports were published more than 30 years ago, and it is possible that their results were different from that of the current Japanese MF cohort for the following reason. Their studies included both dentate and edentate mandibles and did not take any account of the effects of teeth being present. We consider this as an important consideration, in that resorption of the alveolar bone following tooth loss has a major influence on the MF opening. [6] The loss of teeth depends largely on personal circumstances such as, for example, the oral environment of the individual. As a consequence, it is difficult to consider that their results were solely age dependent. The present study was limited to subjects with at least 20 remaining teeth, including posterior molar teeth and, by inference, the results would not be seen to be affected by alveolar bone resorption. For this reason, it is considered that the results of this present study are more indicative of actual age-related changes in MF position.

Several different methods have been used to assess MF position and the location of the mandibular canal in MF, including dry mandibular macroscopic observation, panoramic tomography,[24-26] and cone-beam CT (CBCT).[27-29] However for assessment of the age-related change of the MF opening direction, it was considered that the use of MDCT would be the most appropriate methodology. Using macroscopic observation of MF it is necessary to dissect out the mandible. Not only is this an invasive procedure but it also time intense. Additionally the MF must be identified blindly on the inside of the cortical bone. It is difficult to decide the measurement point and this is likely to increase the error between measurements. By contrast, MDCT is a non-invasive procedure. 3D-CT images are as accurate as direct observation for purposes of measurement,[30] and obviate the necessity for blind searching of the opening direction of the MF. Using panoramic tomography, it is not possible to obtain 3D information, and the MF cannot be viewed from different angles. Ngeow et al.[25] reported a weakness using dental panoramic radiographs in that the visibility of the anterior loop of the mental nerve becomes difficult to visualize due to the decreased calcification of the bone around the MF in older subjects. As a consequence, these panoramic images could not be used in the present study which involved measurement of the two angles of opening of MF in the coronal and transverse planes.

CBCT is commonly used in the field of clinical dentistry and is usually performed with the subject in a sitting position. This can present practical difficulties in research involving cadavers. However MDCT can be performed with the subject in a supine position. Compared to CBCT, MDCT is able to obtain data with less image noise and is suitable for observation of the mandibular bone.[31,32]

In the present study accurate and objective data using MDCT was obtained regarding the position of MF in Japanese subjects. It is accepted that there were limitations to the scope of the study. All of the subjects were Japanese, and the number of cases was small. Further studies with larger numbers of cases, including other races, are needed to clarify the relationship between age and the position of MF.

CONCLUSION

The present study demonstrated that there are age-related changes apparent in MF opening direction of Japanese males. By contrast the present study demonstrated that there are no age-related changes apparent in MF opening direction of Japanese females. In the coronal plane, the opening direction of the MF moves superiorly until the early 50s, after which point, it moves inferiorly. In the transverse plane, it moves anteriorly with age. These observations differ from the results of previous studies. The findings of this present study could be relevant in the field of forensic anthropology as they indicate changes of the position of MF in contemporary Japanese males that may be helpful in the identification of human remains.

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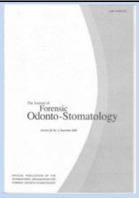

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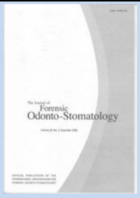

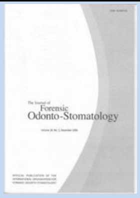

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SECTION AGE ESTIMATION

A Comparative Evaluation Of The Applicability Of Two Adapted Häävikko Methods For Age Estimation Of 5-15 Year Old Indian Children

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The authors declare that they have no conflict of interest.

ABSTRACT
Dental age determination methods that require the assessment of all teeth are tedious and time-consuming. Adapted methods that assess fewer teeth may be more easily applicable. The present study compared the applicability of two adapted Häävikko methods which evaluate seven mandibular teeth (HAM1) and four reference teeth (HAM2) in a population of 5 to 15 year-old Indian children. The HAM1 method underestimated age by -0.17 ± 0.80 years, -0.29 ± 0.83 years and -0.22 ± 0.82 years in boys, girls and the total sample respectively, while the HAM2 method underestimated age by -0.34 ± 0.88 years, -0.51 ± 0.82 years and -0.41 ± 0.86 years in boys, girls and the total sample, respectively. Significant gender-based differences were observed in mean DA-CA with both methods (p < 0.05). While both methods could be used for age estimation of the present population, the HAM1 method was the more accurate of the two.

KEYWORDS: Häävikko method, Adapted Häävikko method, Age estimation, Indian children

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INTRODUCTION

As early as 1935, Schour and Hoffman[1] observed that the pattern of calcification of the dentition under normal conditions acts as a reliable indicator of the pattern of growth. Since then it has been established that dental development by reference to calcification of the developing teeth is an appropriate measure of dental maturity, having high reliability, low variability and resistance to environmental effects, and thereby, allowing for improved prediction of dental maturity.[2-5]

In an attempt to quantify the process of dental maturation from the first traces of cusp mineralization to closure of the root apex, different methods of staging have been suggested, such as the eight-stage, fifteen-stage, sixteen-stage and possibly forty-stage methods of Demirjian, Goldstein and Tanner,[6] Gleiser and Hunt,[7] Moorrees et al.[8] and Nolla,[9] respectively. It should be noted that the methods cited above differ regarding the teeth used for radiographic evaluation; for example, Gleiser and Hunt[7] evaluated the permanent mandibular first molar and Demirjian, Goldstein and Tanner[6] assessed the seven left permanent mandibular teeth (with the exception of the third molar), while Nolla[9] assessed all the permanent teeth in both the jaws.

Häävikko[10] utilized a modified version of the dental developmental stages of Gleiser and Hunt[7] with the number of stages reduced from 15 to 12 (six each for crown and root formation) to study the ages of tooth formation in Finnish children. From data derived by evaluating all the maxillary and mandibular teeth, Häävikko[10] constructed gender-specific tables of age medians and dispersions for each stage of tooth development. Age medians for each tooth assessed were summed and divided by the number of teeth assessed to directly give the dental age. In a later study,[11] the author concluded that it is possible to make reliable estimates of the dental age using only a few specific teeth.

Globally, the few studies testing the applicability of the Häävikko method have reported either age overestimations,[12,13] or underestimations[14-18] or both,[19] using fourteen[12] or seven[13,14] mandibular teeth, four reference teeth[15,16,18] or developing teeth of the left mandible.[19] Studies on Indian populations have been very few with sample sizes ranging from 75 to 660[20-22] and have employed either the original (using all maxillary and mandibular teeth)[20,21] or the adapted Häävikko[22] (using four reference teeth) methods. The dental literature does not contain any reports of comparisons between adaptations of the Häävikko method for applicability in age determination and hence, this study aimed to provide this information using two methods which evaluate seven mandibular teeth and four reference teeth on a population of 5 to 15 year-old Indian children.

MATERIALS AND METHODS

This study was designed as a cross-sectional observational study. Ethical clearance was obtained from the Ethical Committee, Pacific Dental College and Hospital, Udaipur, India (Ref. No. PDCH/13/EC-106). Parents/ guardians had signed an agreement with the dental institution that dental records and radiographs could be used only for research and educational purposes without the possibility of personal identification.

Sampling method: A convenience sampling method was employed, all radiographs were captured during the period from January 2012 to September 2015 of children aged between 5.0 and 15.9 years who had sought treatment at the Department of Paediatric Dentistry, Pacific Dental College and Hospital, Udaipur, Rajasthan, India, and required an orthopantomograph (OPG) as part of the investigation protocol.

Inclusion criteria: Both parents of all the children included in the study were of Indian origin and nationality. Only patients with a documented date of birth and date of capture of the appropriate radiograph in the oral health record were included to facilitate verification of the chronological age (in completed years) for each subject.

Exclusion criteria: Panoramic radiographs showing image distortion due to improper position or movement of the patient during exposure, and incomplete image or lack of clarity resulting from an improper exposure technique were excluded. Also, radiographs were excluded from the study if the patient had any history of surgical/medical treatment or systemic illness with the potential to cause significantly delayed or early development, significant numbers of teeth other than third molars missing either congenitally or due to disease and trauma, malformation of teeth or obvious dental pathology that could affect tooth development.

Final sample: Of the 1303 radiographs collected, 103 did not meet the selection criteria owing to either congenital absence of several teeth (22), lack of image clarity (08) or inadequate information regarding the date of birth (73). Thus, a final sample of 1200 OPGS of 699 male and 501 female Indian children aged 5 to 15 years was selected for the study. The distribution of radiographs by age and gender is presented in Table 1. Radiographs of patients aged 5.0 to 5.9 years were included in age group 5, of those aged 6.0 to 6.9 years in age group 6 and so on. Thus, age group 15 consisted of children aged 15.0 to 15.9 years.

Calculation of chronological age: The dates of birth and of panoramic radiography were obtained from the hospital records. A function of Microsoft Excel was used to calculate the difference between the recorded date of birth and the date on which the panoramic radiograph was made, to obtain the chronological age (CA) in decimal years.

Data collection: All digital radiographs meeting the selection criteria were viewed on the same LCD monitor using a magnifying glass for improved visualization. Each OPG was coded with a numerical ID to avoid examiner bias. Age and sex of the subjects were thus unknown to the examiner. Nomenclature for teeth assessed was assigned according to the FDI system. In Häävikko's adapted method 1 (HAM1), seven mandibular teeth of the left side (excluding the third molar) were evaluated by Häävikko's dental staging method.[10] Once the stage that most accurately described the stage of development of the tooth in question was identified, the corresponding code was assigned to that tooth.

Table 1: Distribution of the study sample by age and gender

Chronological age (years)		Females		Males		Total	
Age group	Age range	N	%	N	%	N	%
5	5.0 - 5.9	24	4.79	23	3.29	47	3.92
6	6.0 - 6.9	39	7.78	40	5.72	79	6.58
7	7.0 - 7.9	46	9.18	58	8.30	104	8.67
8	8.0 - 8.9	50	9.98	58	8.30	108	9.00
9	9.0 - 9.9	55	10.98	78	11.16	133	11.08
10	10.0 - 10.9	55	10.98	100	14.31	155	12.92
11	11.0 - 11.9	40	7.98	82	11.73	122	10.17
12	12.0 - 12.9	55	10.98	91	13.02	146	12.17
13	13.0 - 13.9	57	11.38	82	11.73	139	11.58
14	14.0 - 14.9	59	11.78	58	8.30	117	9.75
15	15.0 - 15.9	21	4.19	29	4.15	50	4.17
Total sample	5.0 - 15.9	501	100	699	100	1200	100

These codes were converted to the gender-specific numerical scores (age medians) of Häävikko.[10] The individual scores were summed and divided by the number of teeth assessed to directly obtain the dental age in years. In adaptation 2 of Häävikko's original method (HAM2), the procedure remained the same with the exception that only four reference teeth were evaluated - 47, 46 (16), 44 and 41 for children aged 0 to 9 years and 47, 44, 13 and 43 for those aged 10 years and above.[11]

Reproducibility of measurements: Two well-trained examiners independently evaluated 100 radiographs using Häävikko's method of dental staging, after a period of mutual calibration without any knowledge of age or gender, in order to allow an analysis of inter-examiner agreement. Ultimately, a single examiner assessed all radiographs. Intra-examiner agreement was assessed by having one examiner re-evaluate the same 100 radiographs after a period of 2 months without any knowledge of gender or age or of the stages assigned in the first evaluation.

Data analysis: All statistical analyses and data management were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA) for Windows and MS-Excel (Microsoft Office 2010). Analyses were made for each gender and age group, and for the total sample. Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to test the normality of the data. As the sample size was less than 30 and having non-normal distribution in some age groups, non-parametric tests were indicated. However, to be consistent across the age groups, both parametric and non-parametric tests were applied. For all tests, a p value ≤ 0.05 was considered statistically significant.

Accuracy of each method of age estimation was determined by mean difference between estimated dental age and the chronological age (DA–CA) for each gender and age group, and the total sample. A positive result indicated an over-estimation, and a negative result indicated an under-estimation of age. Box-plot graphs are used to present the mean DA-CA of each gender and age group, and the total sample, with whiskers indicating the range. Absolute accuracy was determined by means of the absolute differences between DA and CA of girls and boys and the total sample for each method. Paired t test and Wilcoxon Signed Rank test were applied to assess the significance of DA-CA for both methods for each gender and age group, for the total sample and between methods. Independent t-test was employed for intra-method comparisons of DA-CA between genders. The correlation between DA and CA was analysed using Spearman's rank correlation coefficient for each gender and for the total study sample. Inter- and intra-examiner agreements are expressed as percentages. Cohen's kappa coefficient was used to calculate the degree of reliability of these agreements. Regression analyses were performed and gender-specific equations were derived for both the methods.

RESULTS

The mean age (± SD) of the entire sample was 10.75 ± 2.72 years, those of girls and boys being 10.68 ± 2.87 and 10.81 ± 2.60, respectively. Inter- and intra-examiner agreements were 86% and 93% respectively, with Kappa values of 0.81 and 0.90 indicating almost perfect agreement.

In the present study, the mean HAM1 dental ages obtained were 10.39 ± 2.93 years and 10.64 ± 2.80 years for girls and boys, respectively (Fig.1). The mean differences between dental and chronological ages for boys, girls and the total sample (-0.17 ± 0.80, -0.29 ± 0.83 and -0.22 ± 0.82 years, respectively) were statistically significant ($p < 0.05$). Significant differences between mean dental and chronological ages were observed in age groups 6, 7, 9, 10, 11, 12 and 15 for girls and 6, 7, 11, 12, 13 and 14 for boys ($p < 0.05$). In girls, the method underestimated age by -0.03 to -0.64 years in all age groups with the exception of group 14 for which an overestimation of +0.01 years was obtained. In boys, underestimations ranged from -0.15 to -0.61 years in most age groups, with overestimations by +0.04 to +0.36 years in age groups 5, 13, 14 and 15 (Table 2).

Table 2: Comparison of chronological and HAM1 dental ages by gender and age

Gender	Age group (years)	N	Mean age ± SD (years)		Mean DA-CA (years)	p value*	p value#
			CA	DA			
GIRLS	5	24	5.46 ± 0.33	5.43 ± 0.70	-0.03 ± 0.65	0.811	0.808
	6	39	6.57 ± 0.32	6.37 ± 0.58	-0.20 ± 0.49	**0.014**	**0.010**
	7	46	7.52 ± 0.26	7.13 ± 0.82	-0.39 ± 0.87	**0.005**	**0.007**
	8	50	8.51 ± 0.31	8.42 ± 0.73	-0.09 ± 0.73	0.373	0.449
	9	55	9.48 ± 0.30	9.27 ± 0.77	-0.21 ± 0.81	**0.046**	**0.048**
	10	55	10.55 ± 0.32	10.04 ± 0.66	-0.51 ± 0.78	**< 0.001**	**< 0.001**
	11	40	11.44 ± 0.32	10.80 ± 0.77	-0.64 ± 0.91	**< 0.001**	**< 0.001**
	12	55	12.49 ± 0.32	12.00 ± 0.81	-0.49 ± 0.83	**< 0.001**	**< 0.001**
	13	57	13.46 ± 0.30	13.22 ± 0.89	-0.24 ± 1.04	0.087	0.105
	14	59	14.48 ± 0.28	14.49 ± 0.74	0.01 ± 0.83	0.911	0.623
	15	21	15.48 ± 0.27	15.00 ± 0.63	-0.48 ± 0.71	**0.005**	**0.007**
	Total	501	10.68 ± 2.87	10.39 ± 2.93	-0.29 ± 0.83	**< 0.001**	**< 0.001**
BOYS	5	23	5.56 ± 0.29	5.60 ± 0.61	0.04 ± 0.53	0.809	0.843
	6	40	6.52 ± 0.31	6.12 ± 0.61	-0.40 ± 0.61	**< 0.001**	**0.001**
	7	58	7.48 ± 0.29	7.18 ± 0.74	-0.30 ± 0.74	**0.002**	**0.004**
	8	58	8.47 ± 0.29	8.32 ± 0.71	-0.15 ± 0.77	0.136	0.103
	9	78	9.46 ± 0.28	9.30 ± 0.85	-0.16 ± 0.91	0.115	0.098
	10	100	10.45 ± 0.29	10.30 ± 0.79	-0.15 ± 0.87	0.082	0.051
	11	82	11.51 ± 0.30	10.90 ± 0.46	-0.61 ± 0.53	**< 0.001**	**< 0.001**
	12	91	12.44 ± 0.30	11.87 ± 0.68	-0.57 ± 0.76	**< 0.001**	**< 0.001**
	13	82	13.41 ± 0.31	13.77 ± 0.64	0.36 ± 0.68	**< 0.001**	**< 0.001**
	14	58	14.47 ± 0.31	14.83 ± 0.52	0.36 ± 0.62	**< 0.001**	**< 0.001**
	15	29	15.24 ± 0.25	15.30 ± 0.63	0.06 ± 0.70	0.646	0.721
	Total	699	10.81 ± 2.60	10.64 ± 2.80	-0.17 ± 0.80	**< 0.001**	**< 0.001**
Total sample		1200	10.75 ± 2.72	10.53 ± 2.86	-0.22 ± 0.82	**< 0.001**	**< 0.001**

**Paired t test, #Wilcoxon Signed Rank test: $p \leq 0.05$ = significant*

The mean HAM2 dental ages were 10.17 ± 2.74 years and 10.47 ± 2.63 years, for girls and boys, respectively (Fig.2). The mean differences between dental and chronological ages for boys, girls and the total sample (-0.34 ± 0.88, -0.51 ± 0.82 and -0.41 ± 0.86 years, respectively) were statistically significant ($p < 0.001$). Significant differences between mean dental and chronological ages were observed in all age groups ($p < 0.05$) except groups 5, 6 and 10 for girls and 6 and 8 for boys ($p > 0.05$). In girls, the HAM2 method underestimated age by -0.01 to -1.10 years in all age groups except group 5 for which an overestimation of +0.05 years was obtained. In boys, underestimations ranged from -0.05 to -0.74 years in most age groups, with overestimations by +0.28 and +0.05 years in age groups 5 and 6 (Table 3).

Significant gender-based differences were observed in mean DA-CA with both the HAM1 ($p < 0.05$) and HAM2 ($p < 0.001$) methods (Table 4). In girls, the differences between mean DA-CA obtained by the HAM1 and HAM2 methods were significant in most age groups ($p < 0.05$) with the exception of groups 5, 7, 10, 11 and 12 ($p > 0.05$). In boys, significant differences were observed in most age groups ($p < 0.05$) except group 11 and 12 ($p > 0.05$) (Table 5).

Although strong linear correlations between CA and DA were observed for both methods ($p < 0.001$) (Table 6), significantly lower DA-CA values were observed with the HAM1 method compared to the HAM2 method in girls, boys as well as in the total sample ($p < 0.001$) (Table 7).

Regression analyses were performed and the following equations were derived:
For the HAM1 method:
Males: $CA = -0.507 + 1.031 \times DA$
Females: $CA = -0.91 + 0.98 \times DA$
For the HAM2 method:
Males: $CA = 0.175 + 0.953 \times DA$
Females: $CA = 0.39 + 0.915 \times DA$

DISCUSSION

While several methods of dental age estimation have been introduced, some common drawbacks include complicated calculations for obtaining the dental age and increased number of stages that make assessments tedious and age estimations less accurate. The convenience of Häävikko's method lies in the fairly small number of stages that are used to assess dental development and in the simple addition of scores that is required to calculate dental age. Studies testing this method have variously used fourteen[12] or seven[13,14] mandibular teeth, four reference teeth,[15,16,18,22] all maxillary and mandibular teeth[21] or developing teeth of the left mandible.[19] Adapted methods that require the assessment of fewer numbers of teeth would make the age estimation process simpler and less time-consuming. Hence, the present study compared adapted Häävikko methods that utilize seven mandibular teeth and four reference teeth on a sample of orthopantomographs of 1200 Indian children, 501 female and 699 male, aged 5 to 15 years, obtained by a convenience sampling method. This method is preferred by most researchers because it is fast, inexpensive, easy and the subjects are conveniently accessible.

Table 3: Comparison of chronological and HAM2 dental ages by gender and age

Gender	Age group (years)	N	Mean age ± SD (years)		Mean DA-CA (years)	p value*	p value#
			CA	DA			
GIRLS	5	24	5.46 ± 0.33	5.51 ± 0.76	0.05 ± 0.68	0.494	0.875
	6	39	6.57 ± 0.32	6.56 ± 0.82	-0.01 ± 0.62	0.508	0.599
	7	46	7.52 ± 0.26	7.11 ± 1.01	-0.41 ± 1.04	**0.025**	**0.022**
	8	50	8.51 ± 0.31	8.01 ± 1.01	-0.50 ± 0.95	**0.003**	**0.002**
	9	55	9.48 ± 0.30	8.99 ± 0.80	-0.49 ± 0.75	**<0.001**	**<0.001**
	10	55	10.55 ± 0.32	10.32 ± 0.96	-0.23 ± 0.89	0.086	0.159
	11	40	11.44 ± 0.32	11.21 ± 0.65	-0.23 ± 0.61	**0.025**	**0.036**
	12	55	12.49 ± 0.32	11.67 ± 0.63	-0.82 ± 0.70	**<0.001**	**<0.001**
	13	57	13.46 ± 0.30	12.36 ± 0.51	-1.10 ± 0.49	**<0.001**	**<0.001**
	14	59	14.48 ± 0.28	13.75 ± 0.85	-0.73 ± 0.74	**<0.001**	**<0.001**
	15	21	15.48 ± 0.27	14.83 ± 0.75	-0.65 ± 0.69	**<0.001**	**<0.001**
	Total	501	10.68 ± 2.87	10.17 ± 2.74	-0.51 ± 0.82	**<0.001**	**<0.001**
BOYS	5	23	5.56 ± 0.29	5.84 ± 0.64	0.28 ± 0.53	**0.011**	**0.014**
	6	40	6.52 ± 0.31	6.57 ± 0.99	0.05 ± 0.90	0.521	0.888
	7	58	7.48 ± 0.29	7.13 ± 0.89	-0.35 ± 0.83	**0.005**	**0.007**
	8	58	8.47 ± 0.29	8.35 ± 0.89	-0.12 ± 0.88	0.457	0.448
	9	78	9.46 ± 0.28	8.86 ± 0.90	-0.60 ± 0.83	**<0.001**	**<0.001**
	10	100	10.45 ± 0.29	10.00 ± 1.13	-0.45 ± 1.09	**<0.001**	**<0.001**
	11	82	11.51 ± 0.30	11.21 ± 1.09	-0.30 ± 1.05	**0.008**	**0.022**
	12	91	12.44 ± 0.30	12.36 ± 0.71	-0.08 ± 0.68	**0.002**	**0.020**
	13	82	13.41 ± 0.31	13.00 ± 0.64	-0.41 ± 0.62	**<0.001**	**<0.001**
	14	58	14.47 ± 0.31	13.86 ± 0.92	-0.61 ± 0.81	**<0.001**	**<0.001**
	15	29	15.24 ± 0.25	14.50 ± 0.73	-0.74 ± 0.69	**<0.001**	**<0.001**
	Total	699	10.81 ± 2.60	10.47 ± 2.63	-0.34 ± 0.88	**<0.001**	**<0.001**
Total sample		1200	10.75 ± 2.72	10.35 ± 2.68	-0.41 ± 0.86	**<0.001**	**<0.001**

Table 4: Intra-method comparison between genders of mean DA-CA

Gender	N	HAM1		HAM2	
		Mean DA-CA ± SD (years)	p value	Mean DA-CA ± SD (years)	p value
Girls	501	-0. 29 ± 0.83	**0.012**	-0.51 ± 0.82	**< 0.001**
Boys	699	-0.17 ± 0.80		-0.34 ± 0.88	

Independent t-test; p≤ 0.05 = significant

Table 5: Inter-method comparison of mean DA-CA by gender and age group

Age group	Girls					Boys				
	N	Mean DA-CA ± SD (years)		p value*	p value#	N	Mean DA-CA ± SD (years)		p value*	p value#
		HAM1	HAM2				HAM1	HAM2		
5	24	-0.03 ± 0.65	0.05 ± 0.68	0.412	0.250	23	0.04 ± 0.53	0.28 ± 0.53	**<0.001**	**<0.001**
6	39	-0.20 ± 0.49	-0.01 ± 0.62	**0.020**	**0.002**	40	-0.40 ± 0.61	0.05 ± 0.90	**0.002**	**<0.001**
7	46	-0.39 ± 0.87	-0.41 ± 1.04	0.642	0.566	58	-0.30 ± 0.74	-0.35 ± 0.83	0.922	**0.023**
8	50	-0.09 ± 0.73	-0.50 ± 0.95	**0.029**	0.333	58	-0.15 ± 0.77	-0.12 ± 0.88	0.497	**0.014**
9	55	-0.21 ± 0.81	-0.49 ± 0.75	**0.016**	0.235	78	-0.16± 0.91	-0.60 ± 0.83	**<0.001**	0.121
10	55	-0.51 ± 0.78	-0.23 ± 0.89	0.391	0.121	100	-0.15 ± 0.87	-0.45 ± 1.09	**0.013**	0.281
11	40	-0.64 ± 0.91	-0.23 ± 0.61	0.487	0.196	82	-0.61 ± 0.53	-0.30 ± 1.05	0.312	0.189
12	55	-0.49 ± 0.83	-0.82 ± 0.70	0.069	0.166	91	-0.57 ± 0.76	-0.08 ± 0.68	0.358	0.075
13	57	-0.24 ± 1.04	-1.10 ± 0.49	**<0.001**	**<0.001**	82	0.36 ± 0.68	-0.41 ± 0.62	**<0.001**	**<0.001**
14	59	0.01 ± 0.83	-0.73 ± 0.74	**<0.001**	**<0.001**	58	0.36 ± 0.62	-0.61 ± 0.81	**<0.001**	**<0.001**
15	21	-0.48 ± 0.71	-0.65 ± 0.69	**<0.001**	**<0.001**	29	0.06 ± 0.70	-0.74 ± 0.69	**<0.001**	**<0.001**
Total	501	-0.29 ± 0.83	-0.51± 0.82	**<0.001**	**<0.001**	699	-0.17 ± 0.80	-0.34 ± 0.88	**<0.001**	**<0.001**

**Paired t test, #Wilcoxon Signed Rank test: p ≤ 0.05 = significant*

Table 6: Correlation between chronological and dental ages by method

Method	r / p values	Females	Males	Total sample
HAM1	r value	0.962	0.959	0.961
	p value	**< 0.001**	**< 0.001**	**< 0.001**
HAM2	r value	0.855	0.851	0.853
	p value	**< 0.001**	**< 0.001**	**< 0.001**

Spearman's rank correlation coefficient: r = Spearman's rho, p = significant

Table 7: Comparison of accuracy of HAM1 and HAM2 methods

Gender	N	HAM1		HAM2		HAM 1 vs 2				
		Mean DA-CA ± SD (years)	Absolute Difference	Mean DA-CA ± SD (years)	Absolute difference	I – II ± SD (years)	Absolute Difference	95% CI	p value*	p value#
Girls	501	- 0.29 ± 0.83	0.67	-0.51±0.82	0.79	0.22±1.09	0.81	-0.303 to -0.111	**<0.001**	**<0.001**
Boys	699	- 0.17 ± 0.80	0.68	-0.34±0.88	0.76	0.16±1.14	0.86	-0.427 to 0.144	0.330	**<0.001**
Total	1200	- 0.22 ± 0.82	0.71	-0.41±0.86	0.77	0.18±1.12	0.84	-0.391 to 0.053	0.135	**<0.001**

**Paired t test, #Wilcoxon Signed Rank test; p≤ 0.05 = significant*

Unlike the developing maxillary permanent teeth whose radiographic views are often obstructed by bony structures of the maxilla, the teeth of the mandible are quite clearly visible in an some previous OPG. Hence, only the mandibular teeth were evaluated in the present study unlike some previous studies.[6,9] Against the background that it is already well-established that a very high degree of symmetry exists between the teeth of the left and right sides,[6,9,23] only the seven mandibular teeth of the left quadrant were assessed. Third molar tooth germs were excluded from assessment because of the high degree of variability observed in third molar genesis and development.[24,25]

Whilst assessing dental age, it is important to consider the proximity of the estimated age to the actual or chronological age as well as the reproducibility of the age estimation method. In the present study, agreements between and within examiners for Häävikko's method of dental staging were obtained in percentages and measured by Cohen's kappa coefficient. This coefficient is a more robust measure rather than a simple percent agreement calculation, taking into account the agreement occurring by chance.[26] Kappa values for inter- and intra-examiner agreements in the present study were 0.81 and 0.90, respectively. Other studies have reported similar values of 0.84[19] and 0.95,[16] and 0.85[15] and 0.90[19] for inter- and intra-examiner agreements, respectively.

Studies testing Häävikko's method have reported over-estimations of mean age by +0.5 (m) and +1.0 (f) years[12] and by +0.50 (m) and + 0.50 (f) years[13] in Croatian children, and under estimations by -0.94 (m) and -1.59 (f) years,[18] -0.60 (m) and -0.80 (f) years,[14] -0.56 (m) and -0.79 (f) years,[17] -0.09 (m) and -0.23 (f) years[15] and -0.29 (m) and -0.41 (f) years[16] in Malay,[18] Turkish,[14] British Caucasian and Bangladeshi,[17] Bosnian-Herzegovian[15] and Italian[16] children, respectively. Mean over- and under-estimations of +0.07 (m) and -0.19 (f) years, respectively, have been reported in Chinese[19] children.

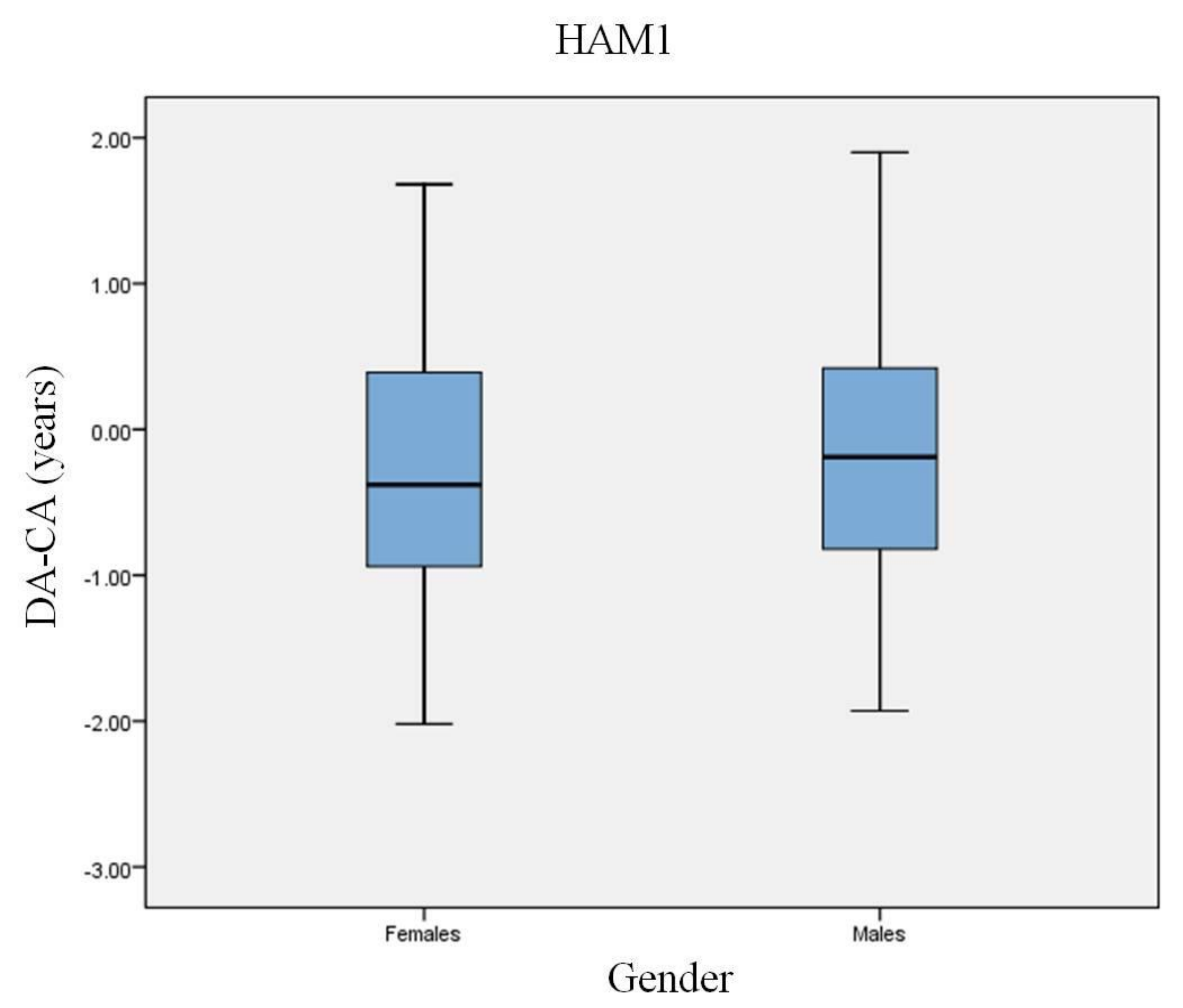

Fig. 1: The mean HAM1 dental ages obtained girls and boys.

Studies on Indian populations have reported mean over-estimations of +0.04 (m) and +0.03 (f) years[20] and under-estimations of -1.78 (m) and -2.12 (f) years[21] and -2.84 (m) and -2.96 (f) years.[22] Two of these studies had very small sample sizes of 75 and 102.[20,21] In the present study, under-estimations of age by -0.17 (m) and -0.29 (f) years were obtained with the seven-teeth method, with significantly higher under-estimations in girls compared to boys. With the four-teeth method, under-estimations of age by -0.34 (m) and -0.51 (f) years were obtained, the under-estimations again being significantly higher in girls than in boys. This gender difference has been attributed to the faster biological and dental maturation in girls, which leads to a higher dental compared to chronological age.[27] However, some other studies[28,29] have reported a higher dental age compared to chronological age in boys than in girls.

In the present study, while strong linear correlations between CA and DA were observed for both methods, the seven-teeth method was more accurate in age estimation than the four-teeth method in girls, boys as well as in the total sample. This would indicate that the accuracy of a method increases when more teeth are examined. However, the accuracy or precision of an age estimating method is also affected by the quality of the reference material (sample), reliability of the method and biological variability in dental development.[12,30] Hence, it is important to accept that no age estimation method can predict the exact age of every individual. While differences between chronological and estimated ages of upto 12 months can be considered to be within normal standards,[31] smaller intervals are desirable.[32] In the present study, mean prediction errors ranged from 2.04 to 6.12 months with both methods.

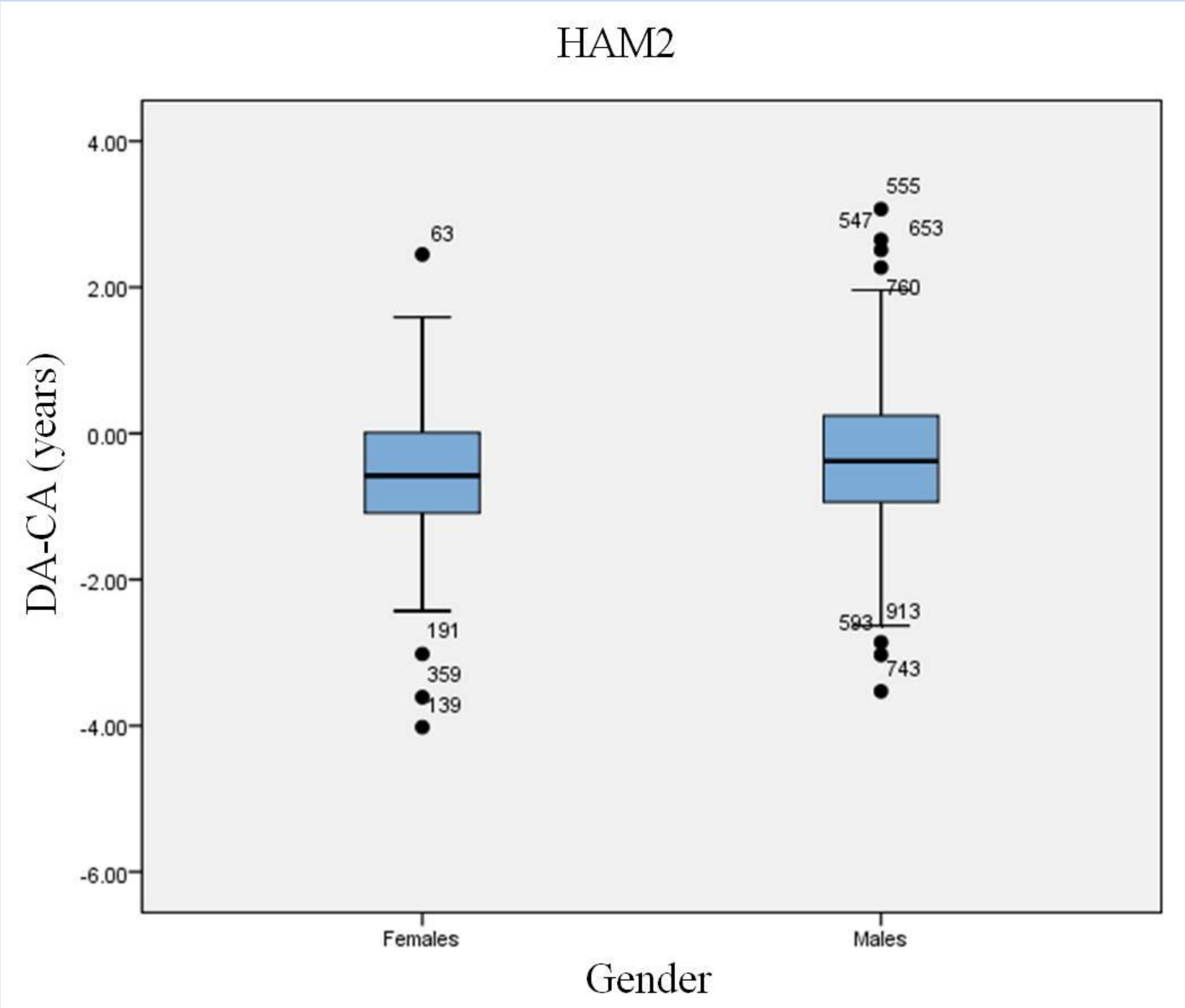

Fig. 2: The mean HAM2 dental ages obtained girls and boys.

CONCLUSIONS

From the results of the present study, it could be concluded that Häävikko's seven-teeth and four-teeth methods underestimated the age of the population studied. While both methods could be used for age estimation of the present population, the former was the more accurate of the two.

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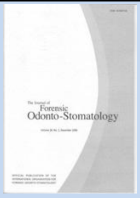

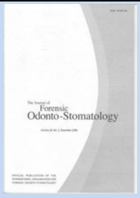

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SECTION AGE ESTIMATION

Reliability and repeatability of pulp volume reconstruction through three different volume calculations

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ABSTRACT
Objective: To test the variability of the volume measurements when different segmentation methods are applied in pulp volume reconstruction. Materials and methods: Osirix® and ITK-SNAP software were used. Different segmentation methods (Part A) and volume approaches (Part B) were tested in a sample of 21 dental CBCT's from upper canines. Different combinations of the data set were also tested on one lower molar and one upper canine (Part C) to determine the variability of the results when automatic segmentation is performed. Results: Although the obtained results show correlation among them(r>0.75), there is no evidence that these methods are sensitive enough to detect small volume changes in structures such as the dental pulp canal (Part A and Part B). Automatic segmentation is highly susceptible to be affected by small variations in the setting parameters (Part C). Conclusions: Although the volumetric reconstruction and pulp/tooth volume ratio has not shown better results than methods based on dental radiographs, it is worth to persevere with the research in this area with new development in imaging techniques.

KEYWORDS: age estimation, pulp volume calculation, image segmentation.

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INTRODUCTION

Since 1950 different methods have been proposed to estimate dental age in adults.[1] Most of them are based on the formation of secondary dentine and the decrease of the pulp chamber size with age.[2,3] Methods that have an invasive approach, (for example aspartic acid racemization and cementum annulation), are not of preference, as they often require the physical damage of the sample.[4] With the evolution of different diagnostic imaging techniques, non-invasive methods for dental age estimation have become the preferred methods,[5] starting with the use of periapical dental radiographs,[2,3] then panoramic radiographs[6] and more recently cone beam computed tomography (CBCT).[7]

Micro-Computer Tomography (µCT) was introduced in the early 20th century[8] to determine age related three-dimensional changes in pulp cavities, from maxillary first premolar teeth. Consistent with early histological research, µCT study's findings indicate that decrease in pulp volume is not linear. It demonstrates a quicker reduction between the 20 and 40 years and then it slows down thereafter. Further research correlating the ratio between pulp and tooth volume with the chronological age, using µCT and linear regression models to estimate dental age in adults, found promising outcomes.[8] However, in light of the high radiation doses associated with µCT, only extracted teeth can be measured in this way.[9] More recently CBCT has been used for dental age estimation calculating the volumes from tooth and pulp chamber. Since then, volumetric reconstruction from CBCT with different software have been reported in various studies for dental age estimation in adults.[10-13] The most recent studies document the use of CBCT from single-[5,13] and multi-rooted teeth.[14]. However, these new methods have not shown superior accuracy to the methods based on dental radiographs.[2,15] It is evident that within each type of software there are a series of sensitivity settings that influences the mathematical approach to measure the baseline volumetric data. These settings clearly have consequences for the outcome and may well be in part the cause of the substantial variation. The primary aim of this study was to test the variability of the volume measurements when different segmentation methods were applied to pulp volume reconstruction. This study is designed to ensure that the underpinning assumptions of a commonly used age estimation approach based on pulp volume calculation are as accurate as possible.

MATERIALS AND METHODS

Ethics approval for this study was obtained from the Human Research Ethics Committee of the University of Western Australia (Ref: RA/4/1/6797). This study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Study sample

A total of 22 anonymized dental CBCT's were used in this study. All of them were recorded with therapeutic purposes, with the consent of the participants, and there was no unnecessary or repeated radiation exposure. All the images were previously unidentified, and age and sex of each individual was the only known information. From these, 57% (n=12) were female, age range 17-63 years, mean age 33.7, and 43% (n=9) were male, age range=15-52 years, mean age 36.7. The CBCT's were obtained from the radiology department of the University of Kebangsaan, Malaysia, and the research group INVIENDO from the National University of Colombia, In both cases the images were obtained using the 9000 3D Extraoral Imaging System (Carestream

Dental, Atlanta, GA, USA) which had a 180˚ rotation and a field of view (FOV) of 50 mm by 37 mm. The radiation exposure parameters were 8-10 mA, 70 Kv, with a slice thickness of 0.076mm. The images were saved in DICOM format. The selected tooth to apply the different segmentation approaches was the upper right canine, or the left when the right was absent (with Federation Dentaire International (FDI) notation 13 and 23) reconstructing one tooth per subject. Only sound teeth, free of any restorations with completed apex formation were included.
Measurement software ITK-SNAP version 3.4 (open source software, www.itksnap.org) and Osirix® (OnDemand 3D software CyberMed Inc, Seoul, South Korea) were used in this study. All the segmentations were completed by a single observer (TYM). All data was collated using Excel (version 2013 Microsoft Redmont, USA) and statistical analysis was completed using R Core Team version 3.1.3 (2015). (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.)

Description of the method

This study tested the comparative outcome of three different ways of estimating the pulp volume,[5,13,14] dental age indicator used in adults. The tests were carried out on the root pulp chamber of upper canines (n=21), to calculate the correlation between automatic[14] and manual segmentation[5] (Part A)(Table 1) and cone shape approach volume calculation[13] (Part B)(Table 2). The final part of the study (Part C)(Table 3) tested the variability of the results obtained with different setting parameter values combinations for automatic segmentation of the pulp canal of one multi-rooted tooth, as previously reported in the literature[14] and one single rooted tooth. (Figure 1)

Part A. Automatic and manual segmentation

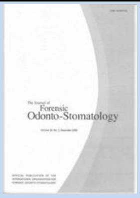
Automatic segmentation was performed using the software ITK-SNAP. (Figure 1. A). Manual segmentation used software Osirix ® and its 2D viewer, (Figure 1. B) which allows the observer to go slice by slice and manually select the boundaries of the space to reconstruct, using the tool polygon. The automatic segmentation process in ITK-SNAP is called seed region-growing. To apply it, the observer sets up a "seed" inside the structure to reconstruct, in this case the pulp chamber. This seed grows and a volume is finally obtained.

Automatic segmentation of the pulp was generated using the same combination of setting parameters through the entire sample: Scale of Gaussian blurring: 3.0, edge transformation contrast: 0.2, edge transformation exponent: 4.0, expansion (balloon) force: 1.0, smoothing (curvature) force: 0.2, edge attraction (advection) force 2.0. In three cases the edge transformation exponent needed to be adjusted to 0.05.

Regression models were run to establish which of the setting parameters would have the most significant influence on the final results. Little changes in the Scale of Gaussian generated the bigger changes in the final obtained values ($p < 0.001$).

With manual segmentation two approaches were used. Firstly, every fourth slice was analysed as for the published method. Secondly, the first and last slice was used as the sample. To analyse the same length of root in the automatic and manual approaches, a length defined in the automatic approach was set for the manual approach.

Part B. Cone shape geometric approximation



The geometric approximation of the root canal to cone is a simple conservative method and has been used for dental age estimation in adults. Using Osirix®, ovals of best fit were formed in the root canal at the cemento-enamel junction (CEJ) level, following the reported method.[13] Secondly, instead of best fit ovals, the boundaries of the pulp canal were drawn using the tool polygon. (Figure 1, C) The root length was measured from the CEJ to the apex, (Figure 1, D) and finally two different volumes of cones were calculated per tooth with a mathematical formula.

Part C. Automatic segmentation and different setting parameters combinations

As observed in part A, it is not possible to apply the same combination of setting parameters values to all teeth with automatic segmentation. Taking into account the advantage of this automatic process[16] twenty combinations of different values for all the setting parameters (Table 3) were used to reconstruct the pulp chamber of one lower first molar (FDI 36, Male 23 year old Malaysian origin, slice thickness 0.2mm) and an upper canine (FDI 23, Male 31 year old Colombian origin). The volumetric reconstruction with each combination was completed twice per tooth. The variation among the obtained volume from the same tooth was calculated.

Statistical analysis

Shapiro Wilk normality test, mean, standard deviation (SD), paired *t*-Test and Pearson correlation coefficient, were calculated to compare the results obtained using the different segmentation methods (Part A), cone shape volume approach (Part B), and the obtained results when different parameters are used to do the automatic segmentation of the same tooth (Part C).

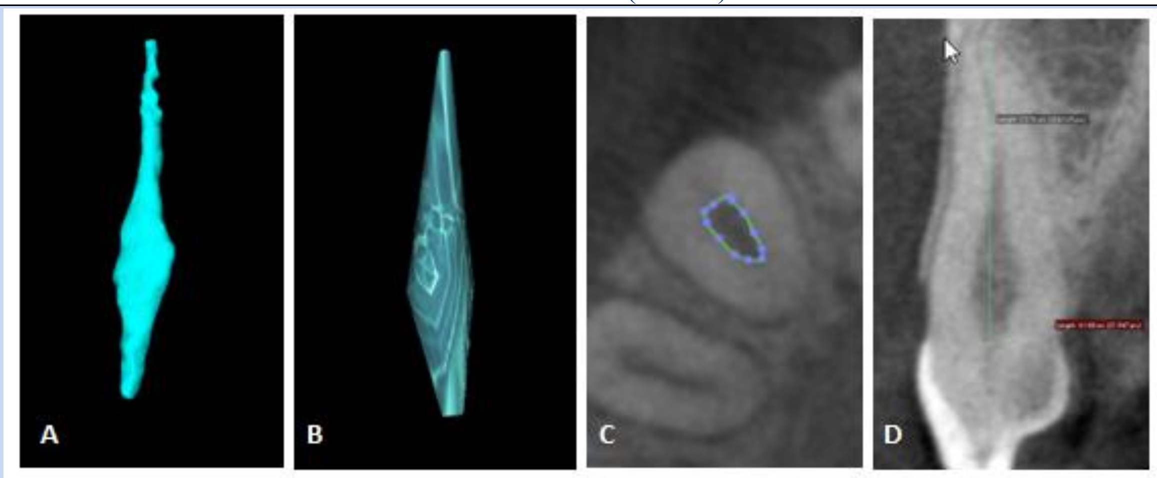


Fig.1: Volumetric reconstructions, upper right canine using different methods
Volumetric reconstruction of tooth 13 from a female individual, 36 years old, using the different methodologies mentioned in this paper. A: Semiautomatic recontruction using ITK-SNAP software. B: Manual segmentation using Osirix ® software. C and D: Cone shape geometric approximation, following the methodology reported by the author doing area measurement of he root canal at the level of the CEJ (D) and length measurement of the root from the CEJ up to the root apex (C), using Osirix ® software.

RESULTS

Part A. Automatic and manual segmentation

Three data-sets were obtained from the volumetric reconstruction of the root canal of the canines using the different methods (Table 1).

When automatic and manual segmentation using each fourth slice were compared, the Pearson's correlation coefficient (r=0.83), shows a greater correlation between them, than between automatic and manual segmentation using only the first and the last slice (r=0.75) or between the two manual segmentation methods (r=0.79). However, this does not mean that manual segmentation using every fourth slice and automatic segmentation produce comparable results. The paired t test shows that there is a statistically significant difference between the means of the results of both manual segmentations and automatic segmentation ($p<0.001$).

When comparing the obtained volumes, it was observed that manual segmentation produced volumes that are larger than those obtained with automatic segmentation. To facilitate the understanding of this difference, a simple mathematical analysis was used, taking the obtained volume with automatic segmentation as 100% of the volume. Then the difference between the volumes obtained with automatic segmentation and both manual segmentation, were used in a rule of three to calculate the percentage this difference represents.

The results of this calculation showed that the volumes obtained using every fourth slice are in average 25% (1%-53%) larger than those obtained with automatic segmentation. Also, that using the first and the last slice are in average 57% (12% to 210%) larger than those obtained with automatic segmentation. The average of the obtained volumes from both manual segmentation methods is in general 41% larger than automatic segmentation.

The volumes obtained doing manual segmentation of each fourth slice were in average 28% larger than those obtained using only the first and the last slice. However, some of them were in average 16% smaller (p>0.05). The mathematical analysis was like the previously described, but in this case, the volume obtained with manual segmentation using only the first and last slice was taken as 100% in the rule of three.

Part B. Cone shape geometric approximation

It was expected to obtain similar area and volume values when using ovals of best fit and the tool polygon following the outline of the pulp chamber. Different than expected, it was observed that with the tool polygon the obtained areas and volumes were noticeably larger (50%) than with the ovals (Table 2). However, there is a strong correlation coefficient between both measured areas using the tools oval and polygon (r=0.84) at the CEJ and the obtained volumes (r=0.88).

When the obtained areas using the two different tools are compared the data show a big variability and a high SD, this effect is diminished once the volume is calculated. In this way, the length of the root is what really determines the final obtained volume.

Part C. Automatic segmentation and different setting parameters combinations

Repeatability of the automatic segmentation for the first and the second repetition for both teeth was evaluated with the paired t test (r=0.7, p=0.99) showing that there is no statistically difference between both repetitions with a 95% level of confidence. The average between the obtained volumes in both repetitions was calculated. The volumes obtained for the molar pulp chamber (FDI 36), ranged from 31.7mm^3 to 53.74mm^3, with a SD of 5.7and a variance of 33.

For the volumetric reconstruction of the canine (FDI 23), the volume value ranged between 29.8 mm^3 to 50.8 mm^3, SD=7.12 and variance of 50.75. The SD for the different parameters was not bigger than 0.6 and the sample variance was not greater than 0.3

Table 1. Volume results of using automatic segmentation (Vol 1), manual segmentation using just the first and last slice (Vol 2) and manual segmentation each forth slice (Vol3), of cone beam computer tomography from upper canines (FDI 13, 23)

Individual	Vol 1 (mm^3)	Vol 2 (mm^3)	Vol 3 (mm^3)
1	10.87	10.6	13.2
2	16.03	17	19.7
3	16.4	20.9	19.8
4	9.309	12.9	14.8
5	5.478	7.1385	8.04
6	11.25	12.5	12.6
7	13.26	25.9	16.3
8	16.72	17	23.2
9	37.01	32.6	41.9
10	16.98	23.4	28.9
11	11.77	19.2	17.7
12	5.857	8.7131	11.7
13	9.107	13.3	28.3
14	17.24	33	29.8
15	12.76	27.5	21.1
16	15.87	16.1	27
17	13.2	19.7	22.6
18	11.61	19.7	16.5
19	24.52	35.3	40
20	15.51	22.1	22.1
21	17.6	29.3	35.5
Mean	14.68	20.18	22.41

DISCUSSION

With the introduction of computer vision techniques for medical imaging and their application in dentistry and forensic sciences, the possibilities to propose methods for age and sex estimation are almost unlimited. Owed to the apposition of secondary dentine, the anthropometric assessment of the variation in the ratio between size of the pulp chamber and size of the tooth is still an acceptable dental age predictor.[14] The aim of this study was to test the variability of the volume measurements when different segmentation methods are applied in pulp volume reconstruction, because although it would be expected that with volume measurements the results for age estimation were more reliable, the literature shows the opposite. To find out an explanation to the lower accuracy of the methods based on volume reconstruction, in this study we tested three different methods doing only pulp volume reconstruction.

Table 2. Cone shape approach results using the tool polygon (Pol) and oval (Oval). Pulp area (in mm^2) at the CEJ level and volume (in mm^3) calculation with the measured root length (in mm).

Area	Area Pol	Area Oval	Root length	Vol Pol	Vol Oval
1	22	20	1.55	11.2	10.2
2	29	30.5	1.46	14.1	14.8
3	28	24.5	1.46	13.6	11.9
4	20	9.85	1.51	10.0	4.9
5	21	16	1.04	7.3	5.5
6	16	13.5	1.56	8.3	7.0
7	46	29	1.86	28.6	18.0
8	37	20.5	1.64	20.3	11.2
9	52	41	1.38	24.0	18.9
10	32	26.5	1.63	17.4	14.4
11	30	17	1.28	12.8	7.2
12	34	16	1.38	15.8	7.4
13	32	22	2.10	22.4	15.4
14	28	14	1.71	16.1	8.0
15	42	28.5	1.79	24.9	16.9
16	27	16	1.71	15.4	9.1
17	25	17.5	1.96	16.2	11.3
18	35	25.5	1.47	17.2	12.5
19	58	44.5	1.73	33.9	26.0
20	39	26.5	1.52	19.8	13.4
21	46	24.5	2.17	33.3	17.7
SD	8.7	10.86		7.45	5.2

The methodology analysed in this study, using CBCT, seems to be no more accurate than those methods based on radiographs for dental age estimation in adults, regardless the sample size of the study which ranges from 19 individuals, analysing only 12 canines[7] to 403 individuals analysing only the pulp chamber of first molars.[14] This is clear when the reported accuracy of the different methods is compared. The reported mean absolute error using automatic segmentation of the first molar's pulp chamber is 6.26 years at best.[14] The cone shape geometric approach of pulp and tooth has a standard error of estimation of ± 11.45 years,[13] and finally, manual segmentation of pulp and tooth, has a prediction interval of ± 12 years.[5]

Table 3. Setting parameter combinations used in part C

Repetition	Scale of Gaussian	Edge contrast	Edge transformation exponent.	Smoothing force	Average molar volume mm^3	Average canine volume mm^3
1	2.14	0.09	4	0.2	38.415	36.58
2	3	0.05	3	0.2	45.775	49.7
3	2.05	0.05	3	0.2	34.525	49.7
4	2.5	0.05	3	0.2	46.075	41.81
5	2.5	0.1	2.5	0.2	49.38	48.87
6	2.5	0.2	2.05	0.2	53.74	48.94
7	2	0.1	2.5	0.2	43.55	39.27
8	2.15	0.09	4	0.2	36.53	40.26
9	2.15	0.09	4	0.3	38.94	39.76
10	2.15	0.09	4	0.35	42	39.38
11	3	0.05	3	0.3	47.29	50.89
12	3	0.05	3	0.35	43.555	48.92
13	3	0.05	3	0.4	44.69	48.9
14	2.05	0.05	3	0.3	38.695	35.98
15	2.05	0.05	3	0.35	38.79	35.89
16	2.05	0.05	3	0.4	38.46	36.04
17	1.5	0.05	2.51	0.2	34.3	31.92
18	1.5	0.05	2.5	0.2	34.955	30.08
19	1	0.1	3	0.2	31.73	29.83
20	2	0.05	4	0.2	36.405	35.10
SD	0.53	0.03	0.59	0.07	5.74	7.12
Variance	0.28	0.001	0.35	0.005	32.94	50.75

There are 3 parameters available in ITK-SNAP software that are not listed, as they were constant for all the reconstructions: Edge attraction force (=2) and step size (=1) and smoothing force which was changed from 1 to 0.5 only for the 18th combination.

Other methods also using automatic segmentation of canines reported a prediction interval of 3.47 years at best.[17] When these reported results are compared with some of the most commonly used adult age estimation methods utilising dental radiographs, such as Cameriere et al.[3] (with reported mean predictor errors of 2.37 years[18] to 11.01 years[19] (tooth/pulp area ratio)), or the method developed by Kvaal et al.[2] (pulp/tooth-linear measurements ratios with reported standard estimation errors of ±9.367 years,[20]) it is possible to observe that the use of CBCT and dental structures volume reconstruction, do not improve the final results for adult age estimation. The relation between the lack of the accuracy of the mentioned methods for age estimation and the different methods for volume reconstruction are presented as follow:

Part A. Manual and automatic segmentation may produce similar results, as observed in this study (r=0.83). Manual segmentation is time consuming, difficult to perform and prone to be influenced by the subjectivity of the observer, and personnel trained in this aspect is necessary.[21] Additionally, it is not accurate enough to estimate the variation of the volume in small anatomic structures, such as the root canal, as observed in this study. Although there are different methods for automatic image segmentation, the first problem faced in the bio-medical area, is the exclusive dependence on gradient or intensity analysis without using anatomical information.[21] In the case of automatic segmentation, with ITK-SNAP software, although the software chooses which pixels will be included in the segmentation, the observer finally decides how to adjust the parameters to control the algorithms in the segmentation, making this method prone to a certain degree of subjectivity from the observers. These parameters may change from one CBCT to another. Unfortunately, in the previous study using this software the values of the setting parameters were not mentioned.[14] In this study it was also observed that although all the CBCT were obtained from machines with the same reference, and under similar exposure conditions (slice thickness, Kv, mA and time of exposure), and the same parameters for automatic segmentation, the interaction of the seed region-growing with the boundaries of the structure to reconstruct may also differ from one CBCT to another. For this reason, it is not possible to follow the recommendations of the ITK-SNAP manufacturer, who recommends to keep the same parameters among different CBCT, and it is neither possible to guarantee the uniformity of the segmentations obtained.[14]

In this study, we also observe that small changes in the values of the setting parameters can generate significant variation in the final obtained volumes with automatic segmentation, even though the segmentation was made on the same CBCT, same tooth and same anatomic region.

Which means that the final volume will be always the same under the same parameters in the same CBCT and anatomic region, but these parameters generally need to be modified from one CBCT to another, and in the same way, small changes in the parameters alter the final volume.

Part B. With the aim of simplifying the measurement of the tooth structure volume, a cone shape geometric approach was proposed (Part B).[13] The developers of this method were aware of the main disadvantages of this proposal: measurements inaccuracy and subjectivity. They also reported that this method tends to underestimate the real volume of the tooth structures (56% to 67%), which in theory would not affect the final pulp/tooth ratio that was calculated to estimate the age.

To test this method, we used two differ approaches for doing the volume calculation. The first, following the published methodology[13] displaying ovals at the CEJ, and a second one selecting manually the outline of the root canal at the CEJ, using the tool polygon. As observed in the results section, despite the difference between both area measurements, at the CEJ, once the volume is calculated the difference decreases. Neither of these two approaches is recommended to carry outpulp volume measurements, , firstly because of the subjectivity of the observer, who only counts with the naked eye to display one or another and secondly, because both bring a distorted representation of the root canal, ignoring the different variations in its internal shape.

Part C. In this study, it has been shown, that there are different parameters that affect the final volumetric reconstruction of any anatomical region. The scale of Gaussian, or blurrier factor[22] was found to be the most relevant when doing automatic segmentation. To increase the value of the Gaussian scale, allows the observer to eliminate certain noises in the image, which affect the "growing of the seed". However, the bigger the value the Gaussian scale is, the more principal structures and details will be lost.[23] This would mean that the initial volume of the pulp chamber will be magnified.[16]

Although automatic segmentation is less prone to the subjectivity of the observer than manual segmentation, in this study we observed that it is not possible to keep the same volume reconstruction parameters for all the CBCT, and that minimum changes may produce significant variations even though they are done on the same tooth. The volume obtained with this tool must be understood as an approximation to the real volume of the structure being measured

Taking into consideration the reported rate of secondary dentin formation is 6.5 µm/year for the crown and 10 µm/year for the root,[24] the analysed methods in this study are not sensitive enough to detect such small dimension changes, generating a big variation of the measurements, which could explain the greater error of pulp/tooth volume based methods for age estimation when compared with simpler methods based on linear[2] or area measurements[3] on dental radiographs.

Previous research in the biomedical area have highlighted the problems related to semi-automatic segmentation, and specifically, to the process that the software uses to select which pixels or voxels to be included in the final volume reconstruction. To overcome this problem, they have elaborated the probabilistic anatomic atlas, which facilitates the segmentation of an organ of interest.[21] When doing the semi-autographic segmentation of teeth and pulp chambers, different obstacles were observed. Firstly, owing to the similar density of dentine and bone and the irregularity of the periodontal ligament, it was not possible to obtain a volumetric reconstruction of the tooth as it is in for the pulp chamber. This is the reason why the automatic tooth volume reconstruction was not analysed. Secondly, owing to the large variety in the shape of the pulp chamber in the apical third of the tooth, there was always a leak of the seed region -growing in to the dentine. To create a probabilistic atlas for dentistry could help to overcome these issues, and help the dentist and forensic dentist to implement more accurate and easier methods for automatic segmentation and volume reconstruction. Thirdly, although the automatic reconstruction of the pulp chamber of molars may be understood as a simple process, it may be more reliable to use other methods for age estimation when using molars.

CONCLUSIONS

Based on the results of this study, it is possible to affirm that the obtained volume measurements with any segmentation method must be understood as an approximation of the measured structure and not as the real volume. In the same way, manual segmentation could produce volume values that are even more inaccurate, and should be avoided in future studies.

Although the volumetric reconstruction and P/T volume ratio has not shown better results than methods based on dental radiographs, it is worth to persevere with the research in this area with an interdisciplinary team to build software that can reliably reconstruct pulp and tooth volumes for forensic purposes.

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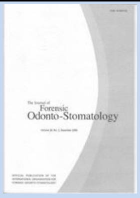

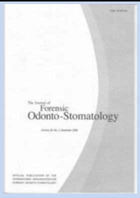

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SECTION IDENTIFICATION

Multiple deaths caused by a fire in a factory: identification and investigative issues

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ABSTRACT

The article presents a case of multiple casualties following a textile factory fire. The incident required a full DVI team similar to large mass-disaster because of the specific operational aspects and identification difficulties. The autopsy results were consistent with death by fire and the toxicological investigations revealed carbon monoxide poisoning in four cases (HbCO% ranging between 88,05 and 95,77), two deaths by cyanide intoxication (with concentrations between 5,17 and 8,85 mcg/ml), and in one case there was a synergistic effect of the two substances (carbon monoxide and cyanide). The identification, carried out in accordance with INTERPOL protocols, encountered serious difficulties in the AM phase primarily due to a language barrier and the lack of any dental or medical information relating to the victims. Secondary identifiers proved to be very useful in corroborating possible identities. As a result of the combined efforts of a team of experts the identitity of each victim was determined and in all cases at least one primary identifier was used in the identification process. The deployment of DVI teams composed of forensic experts from different fields of expertise and well acquainted with DVI procedures, is essential in events involving multiple casualities that may also include foreign victims. The DVI team should intervene not only in PM examinations but also in the collection of AM data for those individuals not accounted for and by helping police in contacting families of missing people.

KEYWORDS: carbonization; body identification; charred bodies; DVI teams; fire multicasualties; tongue protrusion.

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INTRODUCTION

A mass-disaster is defined as *an event which generates more victims at one time than locally available resources can manage using routine procedures. It requires exceptional emergency arrangements and additional or extraordinary assistance.* [1]

In Italy, Magistrates have the responsibility to implement investigations for the identification of victims in cases of mass disaster. They commission case-by-case teams of forensic experts from the police and other Institutions, such as Universities. DVI teams with specifically qualified and trained professionals are not yet available in Italy despite the establishment of DVI section from 2006 [2] by the National State Police. The legal framework on DVI activity and organization is, however, evolving thanks to a new proposal of law that is actually under discussion at Italian Parliament. This law will ultimately implement procedures from Interpol's DVI recommendations and, if implemented, will form the basis for the recruitment and the composition of DVI teams in Italy for the future.

The recovery of burned corpses invariably raises specific forensic issues requiring appropriate intervention in order to both establish the cause of death and the identification of the victims. The forensic pathologist must determine if the victim was exposed to fire before or after death and report possible evidence of homicide, suicide or accidental death. Therefore, a careful examination of the corpse is essential to detect not only vital signs but also to detect any possible injuries contributing to, or causing death. [3, 4]

Fires in a closed environment generally cause severe destruction of the bodies of the victims. Due to the lasting high temperature [5] in these cases visual recognition is not usually appropriate and a scientific identification process through the comparison and reconciliation of primary and secondary identifiers according to DVI Interpol procedures is required. [6, 7].

Any forensic activity, therefore, must be performed with the utmost care, paying very close attention to every aspect of evidence collection at the disaster or crime scene. In cases of advanced carbonization, small or tiny fragments such as teeth may become detached and disregarded as important evidence by personnel not trained in forensics. There is also the ever present risk that certain personal items or body parts could be damaged, misplaced or even lost during victim recovery and transportation to the mortuary. Specific techniques such as fixing sprays or glues can be applied to the body parts prior to removal of the body. [8] During this phase of recovery, the participation of forensic experts is essential in order to obtain a general description of the state of the victims and to record accurately the number of victims and detached body parts. Such methods allow for proper documentation of all personal effects including their positions and locations at the disaster scene. Later these details could provide invaluable information in the identification process. Not only can this information help to identify the victims, but may also lead to an understanding of the possible causation of the deaths and injuries as well as the circumstances of the disaster itself. [9 10]

This paper reports a case of multiple deaths in a textile factory that required the full participation of a DVI team composed of two Forensic pathologists, a Forensic Toxicologist, two Forensic Odontologists and a Forensic DNA expert. Because of specific operational and identification difficulties the DVI team was similar to that deployed for mass-disasters.

In addition, the case was conducted against the background of a serious crime investigation that included the violation of labour and safety laws, building regulations and immigration laws. There were also diplomatic issues, since all of the victims were of Chinese nationality. As a result the case required an extensive police investigation with lasting public and media attention. Hence, forensic experts were not only under pressure to provide prompt and effective identification of the charred bodies, but also to provide relevant data that could prove useful for the Police criminal investigations.

The case highlights the crucial role of the timely deployment of a forensic team, made up of experts as suggested by the DVI Interpol procedures and appropriate to the specific identification and investigative issues of the case. Additionally, this report discusses the reliability of some specific findings used in identifying victims in a closed disaster and complications encountered in PM and AM data collection.

MATERIALS AND METHODS

In September of 2013, a large fire broke out in a textile factory in Prato (central Italy), where a number of illegal Chinese workers were employed.

From the beginning, the case aroused the interest of the public and politicians. The town of Prato, close to Florence, has a long history in textile, a thriving industry. Over the last few decades the town has undergone an economic crisis. It has also seen a rise in Chinese immigration and a number of industries belonging to Chinese entrepreneurs. Typically, these industries employ many illegal immigrants working in sweatshop-like conditions. These employees not only work in the factories but frequently live, eat, and sleep inside them.

The report states the fire night caused by a malfunction of the heating system. It happened at night when many of the occupants were most likely asleep. Some workers near the building's main entrance survived. After the fire was extinguished, firefighters recovered the charred remains of seven bodies, supposedly workers at the same factory.

The Criminal Court of Prato appointed a single Forensic Pathologist who intervened directly at the disaster site. The following day a DVI team was called upon to determine the cause of death and identify the victims. Later a second Forensic Odontologist was called on for quality control as required by Interpol 600's forms.

The factory, with an area of 500 square meters, was made up of a large single space with front access. To the right, there were two rooms; a bathroom and a dormitory. These two rooms were covered by plasterboard. There was a wooden loft, which partially collapsed in the fire along with the roof. In addition to manufacturing equipment the facility housed combustible textile material which fueled the fire. The absence of any sort of accelerant or other suggestive evidence or circumstantial data excluded the possibility of arson.

Figure 1 shows the inside of the building and the position of the victims at recovery. Five of the seven bodies (corresponding to PM 2,3,5,6,7) were found partially buried in the rubble close together at the back of the shed on the right hand side, corresponding to the section of loft that collapsed. Another victim, (PM4) found with a cell phone in the left hand, was on the same side but separated from the others. The last victim, in nightclothes, (PM1), was found in the anterior portion of the loft which was still standing. The victim was near a window covered by a grating with one arm poking through the broken glass of the window.

Because conditions at the factory were dangerous as a result of the fire, the DVI team was not allowed on site. Against this background there was no possibility to identify and recover any remaining body parts that may have been either neglected or overlooked during removal of the bodies.

All forensic activities (AM and PM) for body examination and identification were carried out in the Department of Health Sciences, section of Medical Forensic Sciences at the University of Florence.

Forensic pathologists, odontologists and geneticists collected ante-mortem (AM) data obtained from relatives and acquaintances of the victims. Interviews were conducted with the help of an official Chinese interpreter. During AM sessions, saliva samples were taken from close relatives of the victims and AM data of the missing were registered on AM INTERPOL forms. [11] The postmortem examination started with photographic documentation and full-x-rays of all of the victims or human remains using a portable X-ray machine Corsix Italray®. External examination and full autopsies were then performed according to standard post-mortem forensic procedures as much as was possible given the state of the seriously carbonized remains. [6]

Forensic odontologists conducted dental examinations, including oral photography and intra-oral digital radiography using a Dental Portable X-ray machine Rextar-X® (Posdion Co., Ltd) and Intraoral Digital Dental X-Ray Sensor Sirona Xios®.

Postmortem data were registered on INTERPOL's pink PM DVI forms.

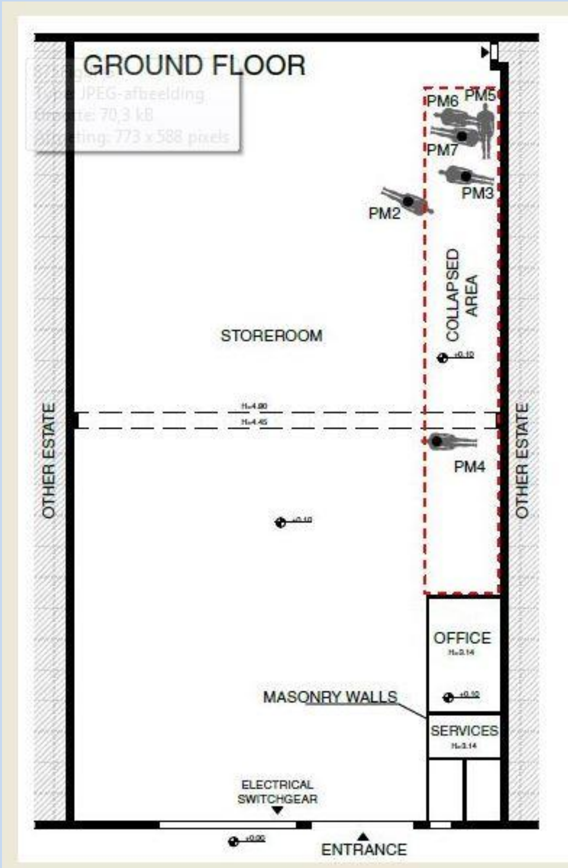
The identification was carried out in accordance with Interpol protocols, [6,7]

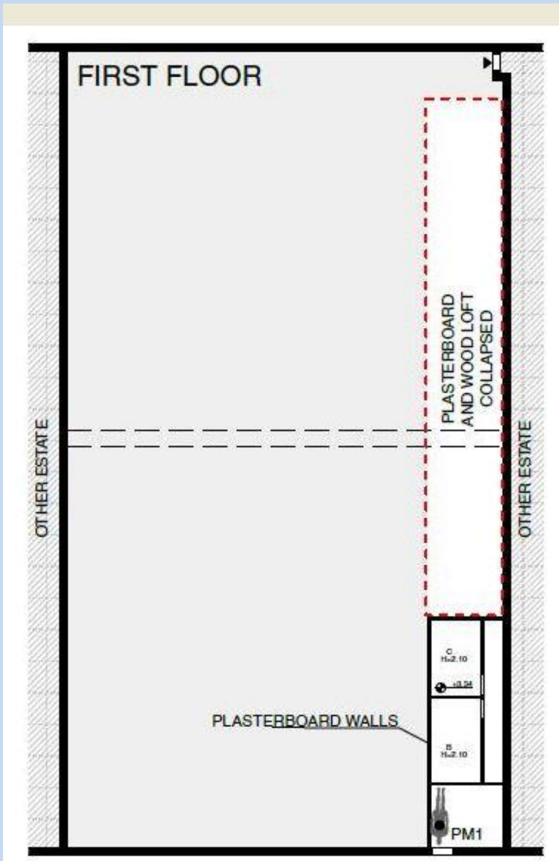
through a careful reconciliation of AM and PM data.

Toxicological investigations were performed on blood samples by gas chromatography-mass spectrometry to investigate amounts of ethanol, psychotropic, hypnotic, toxic or any pharmacologically active substances. In order to assess the cause of death and the status of vitality at the time of the fire, levels of carboxyhemoglobin and cyanide were measured by spectrophotometry.

Genetic investigations were done according to the recommendations of the International Society of Forensic Genetics (ISFG), [12] and of ENFSI (European Network of Forensic Science Institutes)[13]. According to the INTERPOL recommendation [6] samples of muscles and oral mucosa were taken from each victim from anatomical sites that were least damaged by the fire. In cases of severely burned victims one tooth was collected as a possible source of DNA. DNA, extracted through manual and automated procedures [14, 15] from tissue samples, was quantified by Nanodrop method. Genetic profiles were obtained through AmpFlSTR® NGM™ PCR Amplification Kit (Life Technology, USA) and PowerPlex® Y23 System (Promega, USA). Profile comparison was performed using the software Familias®. [16]

Fig.1: GRAPHIC PRINTOUT OF BODY'S POSITION





RESULTS

1. Identification

1.1. P.M. data

Numbering and labeling the bodies (from PM1 to PM7) presented the first major obstacle since each body had two sets of labels and numbers. The first set was done by firefighters at the disaster scene and the second set by mortuary personnel. Complete photo sets taken by the police and the forensic pathologist inside the factory facilitated reconciliation of the two different sets of labels and numbers enabling the original position of the victims to be determined.

Most of the bodies were incomplete and the degree of carbonization was so thorough that facial recognition was possible for only one victim.

Table 1 shows the PM data related to primary identifiers (dental records, fingerprints and DNA) and other autopsy findings as well as relevant circumstantial, autopsy and toxicological findings necessary to ascertain the cause of death.

Amputations and/or charring of the limbs prevented the collection of fingerprints in five of the seven victims. Interpol 600 PM forms were completed with dental data for six of the seven victims despite the high degree of carbonization. Dental date was unavailable for one victim where the splanchnocranium had been completely destroyed. Complete sets of intra-oral X-rays were also taken where possible.

Determining the genetic profile was possible in all cases using soft tissue samples. [17]

Prior to autopsy, full X-rays of the victims were captured; no significant findings were detected.

1.2. A.M. data

Language barriers caused major difficulties during AM interviews. Despite having lived in Italy for several years, many of the Chinese interviewed spoke little or no Italian. The official interpreter was unable to understand all of the various Chinese languages and dialects. In some cases, where verbal communication was unhelpful, odontologists were reduced to asking relatives to sketch rather than describe dental features. Additionally, some of the victims had entered Italy illegally and had no official I.D. making even gathering of basic information difficult. The lack of medical and dental records proved increasingly problematic. Most of the victims seemed to have had dental work done in China but none of the relatives knew whether the missing victims had ever sought treatment in Italy from a physician, hospital or dentist or whether their medical needs had been taken care of within the Chinese community.

No medical or dental records were provided to the Italian authorities by the relatives in China. However, using important information and data (Table 2) gathered from the in-depth interviews with relatives, sufficient material was collected to enable the reconciliation phase. A relative for each of the unidentified victims was available to provide a saliva sample: two brothers, two sisters, one daughter, one son and a mother.

1.3. Reconciliation

The results of the identification activity are summarized in Table 2, which shows the primary identifiers and the secondary means of identification for each victim.

The genetic link between the victim and relatives was assessed in all cases. When the profile of a relative in direct line was provided (three victims), the identification was obtained and expressed in terms of full compatibility; in the other cases a statistical analysis was performed and the results expressed in terms of likelihood ratio. In all cases, a positive association was made confirming, with various degrees of likelihood, and all of the victims were identified (Table 2).

Fingerprints were obtainable in two cases. A positive match was found with two of the missing whose fingerprints had been registered in the AFIS (Automated Fingerprints Identification System). Dental data recounted by relatives suggested possible or probable identification in four cases. For example, PM 2 showed a pin in tooth 22, compatible with a gold crown on an upper incisor, reported by relatives (Table 2); the diastema and the lack of a tooth observed in PM1 matched the anamnestic data provided by AM3.

In four cases the identification was by secondary means of identification, primarily related to anatomical features, such as in the case of PM6, where the absence of the uterus uncovered during the autopsy phase was congruent with a previous hysterectomy reported by relatives.

Carbonization of PM5 was so advanced as to prevent the collecting of any dental data or fingerprints. However, a decorated gold necklace around the victim's neck was identical to the gold necklace in photographs of one of the missing.

The malformation of a nail phalanx on the index finger of the left hand observed in PM1 was initially considered potentially relevant because such malformation is so rare in the general population. Yet it is a typical deformity in sewing machine operators. In fact the malformation was reported by relatives for five of victims and therefore it proved to be inconclusive.

2. Cause of death

As shown in table 1, the toxicological analysis revealed high levels of carboxyhemoglobin (HbCO), ranging between 48.68% and 95.77%, with variable concentrations of cyanides (between 0.29 and 8.85 mcg/ml). In two cases, the death was due to acute cyanide poisoning. Four subjects died of carbon monoxide poisoning. In one case the concentrations of the two substances, although high, were at sub-lethal levels, leading to the hypothesis of a combined synergic effect from the two substances as cause of death.

The levels of ethanol, between 0.15 and 0.71 g/l), were not relevant to death. Nor were any exogenous substances or drugs potentially relevant as a cause of death.

As shown in table 1, in all cases (with the exception of the most severely carbonised body) the autopsy revealed soot in the airway and with variable degrees of soot in the oesophagus. In two cases oedema and redness of the larynx or of the lower airways was observed as a consequence of inhalation of hot gases. Fractures of the skull, together with the presence of blood in the extra-dural spaces observed in the most of the charred bodies found beneath the rubble, can be ascribed to post-mortem events. Similarly, the retraction of the dura and of the brain that was observed is compatible with post-mortem events. The examination disclosed protrusion of the tongue between the teeth in three of the seven victims; in the other four cases this phenomenon could not be determined because of the degree of carbonization of the lower part of the face or of the tongue itself.

DISCUSSION
Identification

The identity of all the bodies was accurately established through primary and secondary identifiers. The forensic experts, however, faced several difficulties arising at different stages of the investigation. Given the severe carbonization of most bodies, fingerprints were retrievable in only two cases, and in one case dental information was missing as a consequence of destruction of the splanchnocranium. Moreover the victims recovered were allocated two different sets of labels and numbers, initially by firefighters at the disaster site and secondarily by mortuary personnel receiving the victims. These problems were caused by the delay in commissioning assistance from a team qualified and familiar with DVI procedures prior to body recovery and labelling.

The case highlights the lack of specific legal guidance currently in force to establish procedures for response, recruitment and operation of DVI teams for both mass disasters and incidents involving multiple casualties. Moreover there is an impelling need to both determine and quantify the qualifications and numbers of experts comprising a DVI team in cases of the necessity of an urgent response.

The impending consideration of a new law concerning mass-disasters and incidents involving multiple casualties currently under examination by the Italian Parliament may result in the appropriate legal implementation of DVI Interpol procedures in Italy. Until a ruling from the Italian Parliament has been granted the current status of an “ad hoc” response initiated by the local Magistrate that encompasses Police and “civilian” specialists (from Universities, hospitals, etc) will be likely to prevail.

Soft tissues for genetic examination resulted adequate for DNA testing from all of the victims, so there was no need for DNA extractions from dental structures. A direct comparison of the DNA from the victims was not possible because there were no biological AM samples available. However DNA from relatives in direct line to the victims was obtainable in some cases. Against this background genetic evaluation was carried out in terms of likelihood. However, amongst the victims, there were neither siblings nor blood relatives available for sampling, so an adequate level of certainty,[18] as stipulated by the ISFG (International Society for Forensic Genetics) was secured.

Of particular significance it is important to state that other identifiers, both primary (dental and fingerprints) and secondary, were used in confirming the genetic results, underlining the importance of a multidisciplinary approach between forensic specialists.[19]

Dental structures are extremely resistant to high temperatures,[20] but in this case three of the victims were so severely carbonized to an extent that the teeth were of no evidential value for purposes of identification. In one case there was complete destruction of the splanchnocranium. The careful collection of teeth and dental fragments and their subsequent insertion into empty sockets enabled high quality digital intra-oral radiographs to be captured for possible comparison with AM dental X-rays.[21, 22, 23,24] Regardless of the lack of AM dental records or X-rays, relatives were able to provide precise dental information that proved most useful in distinguishing one victim from another as in case PM3 (Table 2).

Despite the help of an interpreter, the linguistic barrier hindered interviews of relatives and each interview required in excess two hours. These lengthy interviews involved the entire forensic team and interfered with collecting essential forensic information. It is significant that information gathered from these interviews for example, anatomical features confirmed during autopsy, proved less relevant for purposes of identification than had been predicted.

Ascertainment of the cause of death

Many types of irritating or poisonous gases can result as products of fire combustions in industrial and domestic settings. Among these substances, carbon monoxide (CO) and cyanide (CN) prevail for chemical-toxicological importance, [25] and the literature clearly provides data about the concentrations of these substances in victims of fire. [26, 27]

In the present case, the vast quantity of synthetic textile material, partially recovered in the building, and responsible for the extremely high temperatures reached during the fire, caused at the high amount of hydrogen cyanide and inorganic acids released, typical of the combustion of synthetic polymer. [28]

Toxicological analysis found values compatible with a lethal acute poisoning by carbon monoxide or cyanides, or by a combined action of the two substances.

All of the victims presented at least one sign of vital signs included in the literature in similar cases of death by fire. [29,3].

Protrusion of the tongue between the teeth, observed in charred corpses, is currently being discussed as a sign of vital burning. The exact physiological mechanism involved is still unknown and under discussion. Bernitz [30] attributed it to a reflex laryngospasm induced by air temperatures of >150°C and hyperventilation or the heat-related shrinkage of the soft tissue of the neck. [31] This latter mechanism seems to also be responsible for the intramuscular bleeding of the tongue found in victims of fire and especially associated with low levels of COHb, [32] which other authors have attributed, instead, to a possible acute hemodynamic disturbance in the head including the brain (cranial congestion) during the process of dying by fire. [33] Some authors [34, 3] reported evidence supporting the value of this sign as an additional indicator of vitality; others criticize this assertion, [35, 36] highlighting the absence of a physio-pathological explanation thus supporting it as a "vitality sign". [37] A recent article [38] reported the finding of tongue protrusion in two bodies burnt after death and the protrusion of the tongue is described as a possible result of the genioglossus retraction due to heat.

All of the victims showed evident signs of vital burning and protrusion of the tongue between the teeth was detected in all the victims except for those whose skull was largely destroyed. However the evidence of tongue protrusion as a vital sign in this report is inconclusive.

The presence of blood in the extradural space, the retraction of the dura and brain of reduced dimensions was observed at autopsy in more than one victim. These findings are described in the literature as possible post-mortal phenomena in the case of exposure to prolonged heat affecting the head. Moreover the increased brittleness of the charred bone and the steam pressure within the skull can also be the cause of the fractures to the cranium [39] observed in two of the victims (PM3 and PM6). These features were observed in those victims with severe burning found together under the rubble of combustible material and consequently more exposed to prolonged higher temperatures. The two victims found near the loft that was used as the dormitory area located in the right posterior area of the building demonstrated the maximum degree of burning and incineration. This area would correspond to the area where the fire broke out, most likely while the victims were asleep.

The autopsy and circumstantial findings suggested that the victims with the least damage (PM1 and PM4), presumably realized the presence of fire, but could not escape. It was hypothesized that the security exits and the stairs for reaching the ground floor and the main door were obstructed by either material or by fire itself. Firefighters confirmed that the stairs were made of wood and located in the right posterior area of the building where the fire started. Data concerning the position of the bodies and the circumstantial details confirm the importance of accurate post mortem investigations for evidence-based reconstruction of fire scene and casualties [40]. It is significant that this evidence resulted useful during Court proceedings in establishing criminal and civil liability for both the factory owner and the employer of the victims, both parties being responsible for worker security and the surveillance of building security.

CONCLUSION

The report is a typical multi-casualty case that implies procedures and deployment of DVI personnel similar to mass-disasters due to specific forensic issues and the judicial demands for timely and effective reports on the cause of death and the identification of victims. The disaster occurred inside the largest Chinese community in Italy and some specific difficulties affected the AM data collection and body recovery at the site of the fire.

Thanks to the deployment of a DVI team composed of specialists from different fields of expertise, certain procedural mishaps were avoided. This case provides some important and meaningful lessons:

Site of disaster. The intervention of non-forensic personnel including a forensic pathologist not qualified in DVI procedures produced mislabeling and cross-numbering of victims that resulted in a time-consuming specific reconciliation process. It is probable that tiny but relevant body parts were not recovered or collected at the disaster site.

Autopsy findings and toxicology analysis support the conclusion that the death of the victims was caused by carbon monoxide and/or cyanides intoxication. Our data supports the conclusion that tongue protrusion co-exists with clear signs of vital burning, yet there is no compelling evidence to exclude tongue protrusion is a post-mortal phenomenon.

The collection of AM data from ethnic communities or from developing countries is challenging because of significant language barriers and the lack of AM data. Nonetheless, despite the bare minimum AM data available in this case, a systematic collection of PM data offered meaningful reconciliation of primary and secondary identifiers for all of the victims.

The activities at the scene of the fire as well as the findings from the PM examinations of the victims made a significant contribution to reconstruction of the circumstances of the disaster and to the investigation by the public prosecutor.

This case supports the conclusion that the establishment of DVI teams, guidelines and regulations for recruitment/deployment of forensic experts who are acquainted with procedures, codes, forms and issues connected with DVI activity are an urgent necessity for Italy.

Table 1: Autopsy findings and primary identifiers for bodies; relevant circumstantial, autopsy and toxicological findings to ascertain cause of death.

BODY	POSITION ON THE DISASTER SITE	SEX (Morfological)	Sex (DNA)	EXTERNAL EXAMINATION: STATE OF THE BODY	AUTOPSY FINDINGS	TONGUE THRUST OUT/CLAMPED BETWEEN THE TEETH	DNA (sample)	FINGERPRINTS	ALCOHOL g/l	OTHER TOXIC SUBSTANCES or DRUGS	HbCO %	CYANIDES mcg/ml	CAUSE OF DEATH
PM 1	Anterior portion of the loft (not collapsed) near a window, closed by a gratin	Male	Male	complete, not charred; injury to head, vital injury to right hand	deformity of the distal phalanx of the second finger of the right hand; soot and food material into the trachea and larynx	Yes	Psoas muscle/buccal mucosa	Yes	0,46	no	48,68	8,85	acute cyanide poisoning
PM 2	right rear side of the shed, partially buried under the rubble of a collapsed loft	Male	Male	advanced carbonization, lower limbs missing	soot in the airway and esophagus, pulmonary edema, extradural blood	impossible to assess	Psoas muscle, blood/buccal mucosa	No	0,15	no	61,63	5,17	acute cyanide poisoning
PM 3	right rear side of the shed, partially buried under the rubble of a collapsed loft	Male	Male	advanced carbonization, incomplete, missing: upper right limb, left hand, lower right limb, left foot	soot in the airway, fracture of the skull, extradural blood, shrunk dura, reduced brain volume, pulmonary edema	impossible to assess	Blood, muscle/buccal mucosa	No	0,54	no	81,23	3,63	acute cyanide and carbon monoxide poisoning
PM 4	Right rear side of the shed, far from the others	Male	Male	advanced carbonization, complete	abundant soot in the airways, edema and redness of the larynx, pulmonary edema	Yes	Psoas muscle, blood/buccal mucosa	Yes	0,33	no	95,77	1,25	acute carbon monoxide poisoning
PM 5	right rear side of the shed, partially buried under the rubble of a collapsed loft	Female	Female	advanced carbonization, missing: upper and lower limbs, maxillary bones, teeth, splanchnocranium	absence of organs of the neck, presence of uterus	impossible to assess	Gluteal muscle, blood	No	0,71	no	94,4	0,93	acute carbon monoxide poisoning
PM 6	right rear side of the shed, partially buried under the rubble of a collapsed loft	Impossible to assess	Female	advanced carbonization: missing upper and lower limbs	soot in the airway and esophagus, fracture of the skull, exposure of the abdominal organs	Yes	Muscle, blood, costal bone/buccal mucosa	No	0,18	no	94,89	2,59	acute carbon monoxide poisoning
PM 7	right rear side of the shed, partially buried under the rubble of a collapsed loft	Male	Male	advanced carbonization: missing upper and lower limbs	soot in the airway and in the esophagus, extradural blood, shrunk dura, reduced brain volume	Impossible to assess	Psoas muscle and blood/buccal mucosa	No	0,42	no	88,05	0,29	acute carbon monoxide poisoning

Table 2: Identification sheet reporting comparison of AM and PM data

REPORT	DNA	FINGERPRINTS	DENTAL RECORDS (MATCH AM-PM)	SECONDARY MEANS OF IDENTIFICATION (MATCH AM-PM)	ID SUMMARY
PM1- AM3	Direct line relative	Yes	diastema of 0.5 mm between 13 and 14, and a missing tooth in the right upper jaw, both reported by relatives	deformity of the distal phalanx of the second finger of the right hand	DNA FINGERPRINTS DENTAL Secondary identifiers
PM2- AM7	1: 4,6 x E^{+12}	-	finding of a pin in the tooth 22 in accordance with reported presence of gold crown in the left upper jaw	No inconsistencies, nothing relevant	DNA DENTAL
PM3- AM1	1: 9,8 x e^{+5}	-	periapical bone loss – fistula involving 36 and 16, in agreement with recent reported intake of antibiotics for "teeth". Endodontic therapy and metal-ceramic crown on central incisor (11), with pulp chamber compatible with a dental trauma occurred in child/adolescenthood.	No inconsistencies	DNA DENTAL
PM4- AM2	1:2,8 x e^{+6}	Yes	lack of all third molar and cross bite of 22 with 32-33, compatible with reported retro position of upper incisor.	presence of dorsal nevus, reported by a relative	DNA FINGERPRINTS DENTAL Secondary identifiers
PM5- AM4	Direct line relative	-	Not possible because of the lack of PM dental data	gold necklace recognized by husband	DNA Secondary identifiers
PM6- AM6	Direct line relative	-	Not possible because of the absence of AM dental data	absence of the uterus, consistent with prior hysterectomy (reported by relatives)	DNA Secondary identifiers
PM7- AM5	1:7629	-	Not possible because of the absence of AM dental data	No inconsistencies	DNA

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