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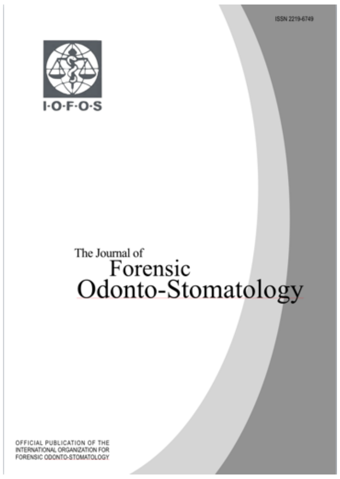

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

This Journal is a peer-reviewed on line publication of papers which should be broadly classifiable as original research, reviews, case reports or news (selected).

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Special Issue

Triennial International Organization for Forensic Odonto-Stomatology Conference,

Leuven, Belgium 2017

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EDITORIAL

Against the background of the rapidly approaching date for the start of the triennial IOFOS International Conference on Forensic Odontology 2017, Leuven, Belgium, a request for presentations, either oral or poster, and corresponding research papers was sent out. The response to this request was beyond expectation, demonstrating that the field of forensic odontology is both global and diverse particularly in terms of research projects. The vast majority of forensic odontologists take on their forensic work in addition to existing clinical commitments. The inference is that the field of forensic odontology is more of a passion and a calling rather than merely an occupation or profession. This is borne out by the fact that the majority of the 150 international delegates attending the conference in Leuven will be present by virtue of their personal financial commitment to attend. This personal commitment will inevitably result in an interest to further progress the discipline of forensic odontology particular in terms of contributions to future research and best practice.

Thank you to all of the presenters and the authors for sharing their work and experiences. The common theme is one of a creative mind and a critical approach. This is evident in all of the contributions and will hopefully serve as an example to inspire and motivate all forensic odontologists particularly our new or younger colleagues. These and future odontology projects will hopefully arouse the interest of a multi-disciplinary audience including among others, forensic scientists, dentists, medical doctors, lawyers, magistrates, non-governmental organization members, and the police. "Joined up" forces are needed for an optimal approach in every case.

Striving to achieve quality assurance will be a highlight of the 2017 conference. New IOFOS working groups were set up in June 2016 to review the previous IOFOS quality assurance recommendations established in 2008. The result of each IOFOS working group will be presented and discussed throughout the 2017 conference in Leuven.

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Journal of Forensic Odonto-Stomatology

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ORIGINAL ARTICLES

A systematic review of odontological sex estimation methods

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KEYWORDS

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dental identification,
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ABSTRACT

Background: In human identification sex estimation plays an important role in the search for ante-mortem data.

Aim: To systematically review studies describing and testing/validating methods of odontological sex estimation. The set research question was: What odontological sex estimation method is the most accurate?

Materials and methods: An electronic search until November 29th 2016 was performed in 5 databases: MEDLINE/PubMed, Cochrane, SciELO, LILACS and Grey literature. The PRISMA guidelines were used. Studies were assessed and included based on the reported data. In particular data criteria were set regarding the considered population, sample size, age range, sex estimation method, type of statistical analysis and study outcome. The extracted data enabled to classify the included studies. Meta-analysis was used to compare the study outcomes per obtained study group.

Results: The established search string detected 4720 studies. 103 were considered eligible after review of title, abstract and full-text. The odontological sex estimation methods were classified based on dental metric and non-metric measurements (n=65), cephalometric analysis (n=13), frontal and maxillary sinuses (n=5), cheiloscopy (n=4), palatal features (n=3) and biochemical analysis of teeth (n=13). Teeth measurements for sex estimation were mainly performed on casts (n=34), followed by skeletal remains (n=13), medical imaging (n=5), intraoral measurements/photography (n=4), and cascades of the above (n=4).

Conclusion: The variety of published odontological sex estimation methods highlights the importance of sex estimation in human identification. Biochemical analysis of teeth proved to be the most accurate method, but in forensic practice, a need to select the most appropriate evidence based odontological sex estimation method exists.

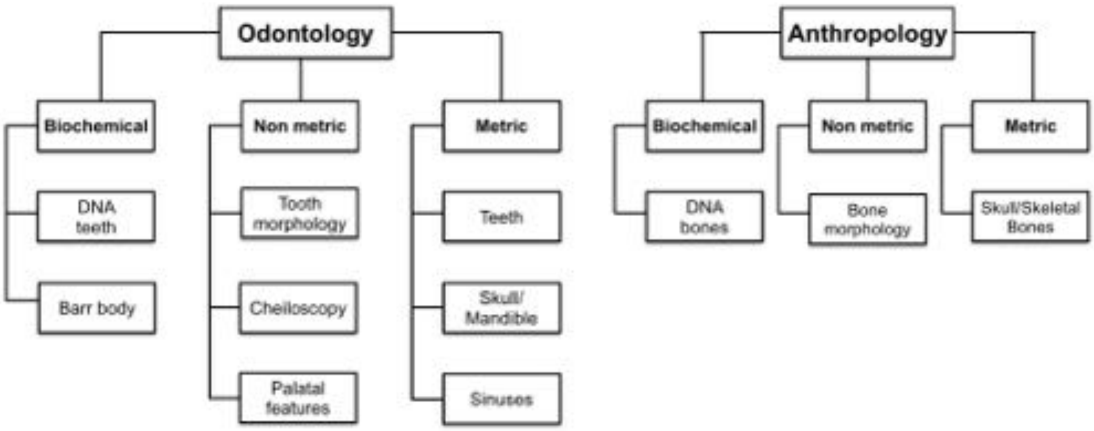
INTRODUCTION

Age, sex and race are defining characteristics for every human individual. In forensic context, sex estimation is an essential part of human identification. Predicting the sex simplifies identifications because missing persons of only the estimated sex need to be considered. Subsequently sex specific age estimation can be performed[1, 2].

Sex estimation is indispensable in diverse forensic disciplines - forensic medicine, forensic odontology and forensic anthropology.

Sex estimation is mainly required for identification of skeletal remains and body parts. Odontological and anthropological methods are used for estimation of sex, both including different metric and non-metric variables and biochemical analyses (Figure 1).

Fig.1: Odontological and anthropological sex estimation methods.



Odontological methods are based on the sexual dimorphism in morphological and metrical features of teeth[3-17] and adjacent structures (lips[18, 19], palate[20, 21], mandible[22], sinuses[23, 24]), and also in biochemical structure of tooth materials[25-27].

Anthropological methods are using morphological features and measurements of skeletal bones (skull, hip, sacrum, scapula, clavicle, sternum, humerus and femur mainly)[25, 26, 28, 29], as well as biochemical analyses of different skeletal materials[25-27].

Divers parameters can be studied for sex estimation in both odontology and anthropology (Figure 2 and Figure 3).

The objective of this systematic review was to analyse publications of odontological sex estimation methods or combinations of these methods.

The set research question was: What odontological sex estimation method is the most accurate?

This was established according to the PICO format as follows:

- Participants/population: human populations used to establish or validate odontological sex estimation methods.
- Intervention: odontological sex estimation methods.
- Comparison of the studied populations and sample size, the considered age range, the sex estimation method established or used, the type of statistical analysis and the study outcome(s).
- Outcome: evidence on the accuracy of the considered odontological sex estimation method(s), isolated or in combination.

Fig.2: Theoretical list of parameters for odontological sex estimation

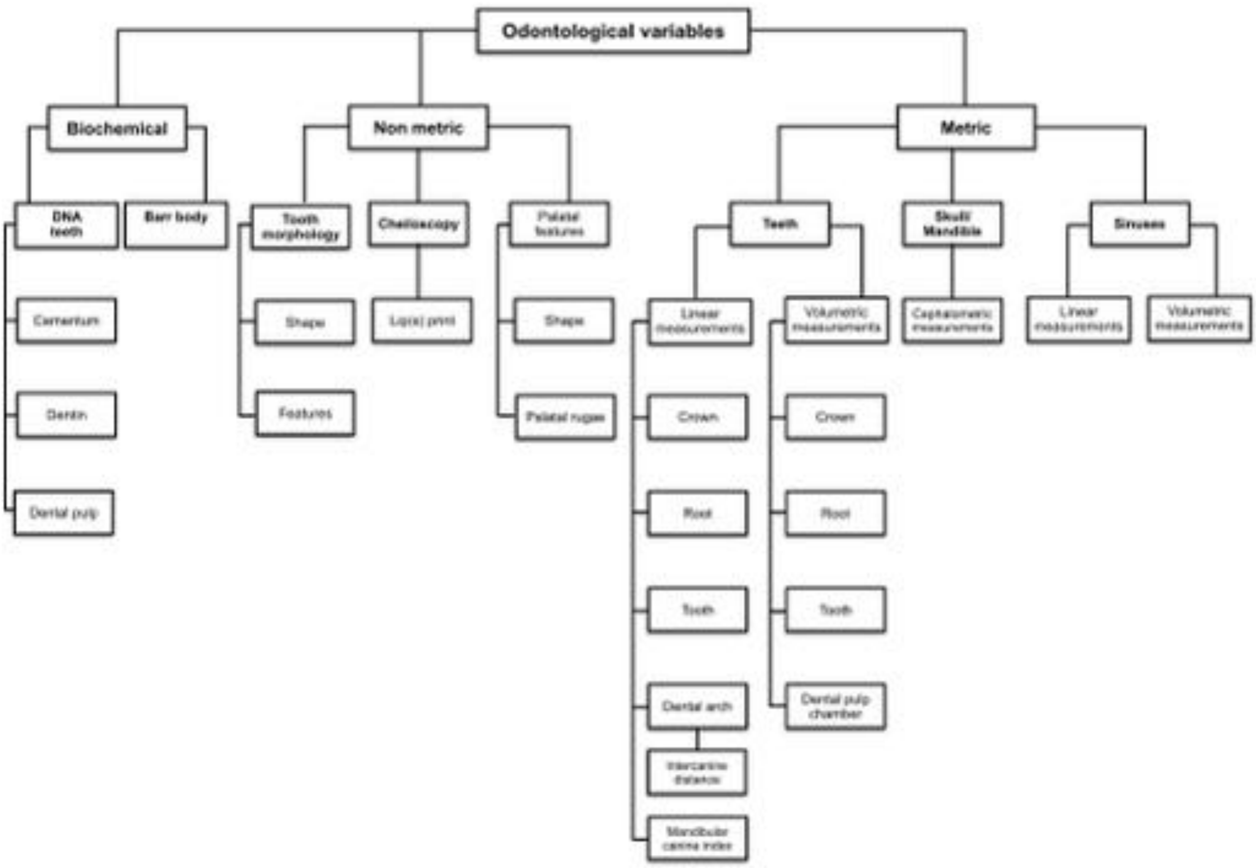
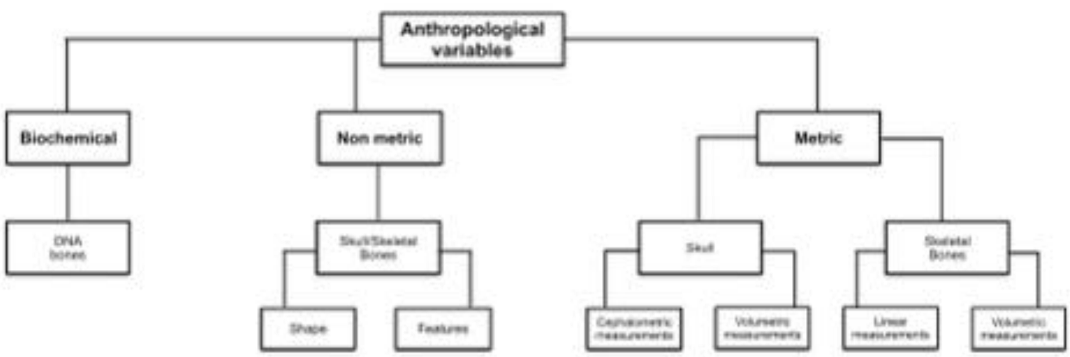


Fig.3: Flow chart of possible parameters for anthropological sex estimation



MATERIALS AND METHODS:

An electronic search until November 29th 2016 was performed in 5 databases: MEDLINE/PubMed, Cochrane, SciELO, LILACS and Grey literature. Keywords related to the study aim and included in the search string were: dental sex estimation methods OR sex estimation by teeth OR dental sex estimation assessment OR dental sex determination methods OR sex determination by teeth OR dental gender estimation methods OR gender determination by teeth.

The Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines were used[30].

The inclusion and exclusion criteria were established by the authors, as shown in Table 1.

Table 1 - Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Studies describing, establishing and validating odontological sex estimation	Studies on anthropological sex estimation
No restriction for sample size, age group and population origin	Studies without an abstract
Studies providing sex estimation outcomes and/or comparing outcomes	Presentations submitted to conferences, only published in related abstract books
No language restrictions	

Based on the title and abstract information a first selection of studies was performed (Figure 4). Next the selected articles were read in full text, to check for their eligibility.

A list of excluded studies was kept. To avoid selection bias reviewing was performed by two of the authors independently, in the case of disagreement between them a consensus was made.

Data extracted, included author(s) and year of publication, studied population, sample size, age range of the sample, used sex estimation method, parameter(s) used, materials examined, and sex estimation outcome(s). Accuracy of sex estimation was established as outcome and extracted from each study.

The quality of the studies included in the systematic review was assessed based on the criteria outlined in Table 2. Comparison between studies was based on a score calculated as 1 per question answered with yes and 0 per question answered with no. The higher the score, the better the scientific quality of the study.

Table 2 - Criteria for quality assessment of studies

Criteria
Was the research question or objective clearly stated?
Was the study population clearly specified and defined?
Were inclusion and exclusion criteria for subjects included in the study sample: • pre-specified? • applied uniformly to all participants?
Were the study parameters clearly defined?
Were the outcome measures: • clearly defined? • validated? • reliable (intra/inter observer)?
Were study bias discussed, related to: • selection bias? • analytical bias?
Did the study have ethical clearance?

The studies were classified based on biological, metric and non-metric odontological sex estimation methods. Descriptive comparison was used for data analyses. Outcome(s) comparisons were made within the same group and also between groups.

Meta-analysis was performed using Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014. Mantel–

Fig.4: Systematic review and meta-analysis flow diagram for the identification of studies assessing odontological sex estimation methods. (n = number of studies)

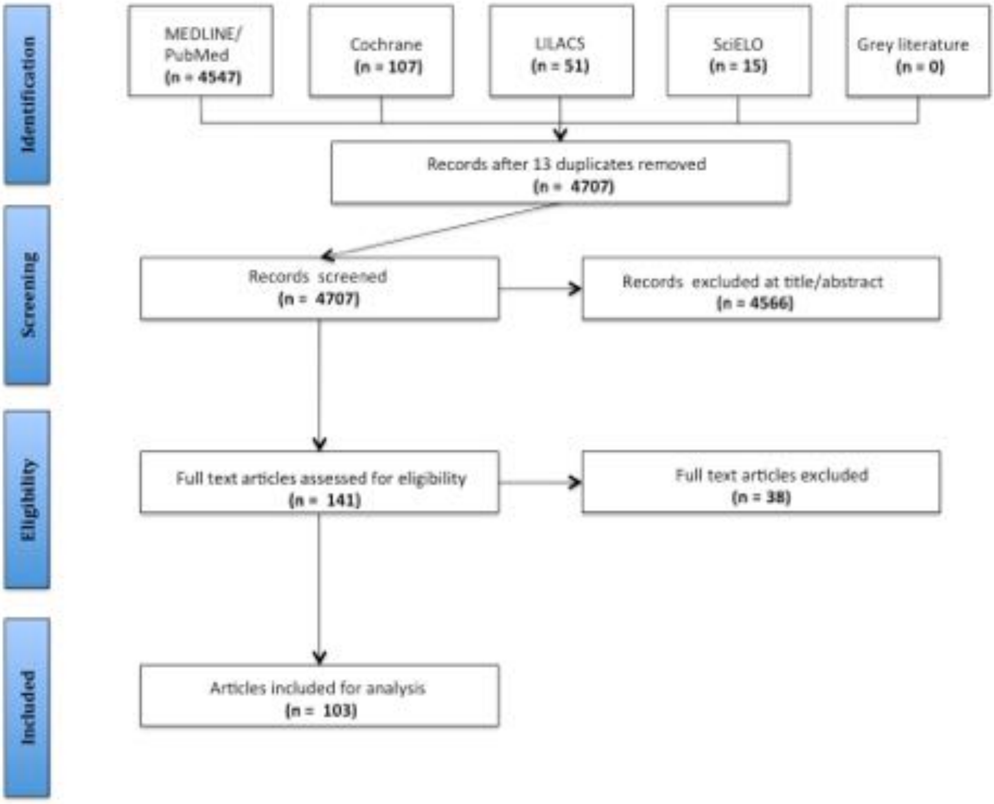
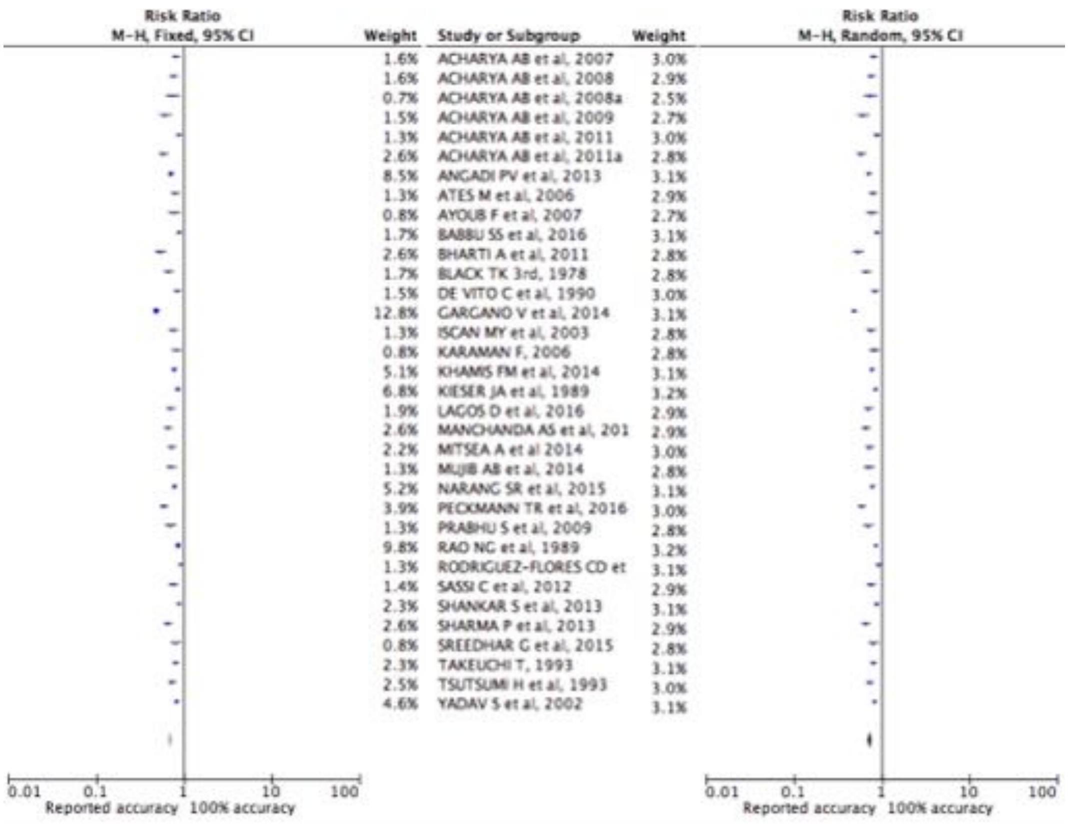


Fig.5: Meta-analysis on the accuracy of sex estimation studies based on odontometric measurements on casts. (M-H = Mantel–Haenszel test, CI = confidence interval)



Haenszel test was used to determine Risk Ratio (RR) using fixed effect (FE) and random effect (RE) models. The analysis was performed within each subgroup of selected studies (Table 3-9).

The influence or "weight" of each study within the subgroup on the overall results was determined by the study's sample size. The bigger the sample size, the greater the weight of the study (e.g. Figure 5).

Table 3 - Sex estimation based on odontometric measurements performed on dental casts

Study	Included Subjects	Age Range	Examined Teeth	Examined on	Method of measurement	Origin Population	Sex Estimation Accuracy	QAS
	Total, M/F*	**(years)**	**(FDI)**				**(%)**	
BLACK TK 3rd, 1978	133 (69M/64F)		51,52,53,54,55,81, 82,83,84,85	Casts	Odontometric	American Whites	63.9-67.7	4
KIESER JA et al, 1989	533 (265M/268F)		maillary permanent teeth	Casts	Odontometric	Lengua Indians/ Negroes/ Caucasian	76.7-93.5	7
RAO NG et al, 1989	766	15-21	33,43	Casts	Odontometric	Indian	84.3-87.5	6
DE VITO C et al, 1990	120	children	all deciduous teeth, 16,26,36,46	Casts	Odontometric	Canadian	76-90	6
TAKEUCHI T, 1993	180	infant/ adults	deciduous/ permanent teeth (excluding I2, PM2, M2, M3)	Casts	Odontometric	Japanese	67.5-93	7
TSUTSUMI H et al, 1993	194 (96M/98F)	3	deciduous teeth maxillary/ madibular one side	Casts	Odontometric	Japanese	67-78.6	6
YADAV S et al, 2002	360 (180M/180F)	15-21	33,43	Casts	Odontometric	Indian	81-83.3	6
ISCAN MY et al, 2003	100 (50M/50F)	average 21	21,22,23,24,25,26 ,27,31,32,33,34,35, 36,37	Casts	Odontometric	Turkish	73-77	7
ATES M et al, 2006	100 (50M/50F)	20-29	all teeth (excluding M3)	Casts	Odontometric	Turkish	76-81	6
KARAMAN F, 2006	60 (30M/30F)			Casts	Odontometric	Turkish	78.3-85	7
ACHARYA AB et al, 2007	123 (65M/58F)	average 22.6	all teeth (excluding M3)	Casts	Odontometric	Nepalese	67.9-92.5	7
AYOUB F et al, 2007	60 (30M/30F)	18-25	32,33,42,43	Casts	Odontometric	Lebanese	63.3-90	7
ACHARYA AB et al, 2008	123 (65M/58F)	19-28	all teeth (excluding M3)	Casts	Odontometric	Nepalese	69.8-81.1	4
ACHARYA AB et al, 2008a	53 (31M/22F)	19-28	all teeth (excluding M3)	Casts	Odontometric	Nepalese	62.3-83	4

RODRIGUEZ-FLORES CD et al, 2008	98 (50M/48F)	5-7	all deciduous teeth	Casts	Odontometric	Argentinian	90.9-93.7	7
ACHARYA AB et al, 2009	117 (63M/54F)	19-28	33,43	Casts	Odontometric	Nepalese	50.8-70.7	5
PRABHU S et al, 2009	105 (52M/53F)	19-32	all teeth (excluding M3)	Casts	Odontometric	Indian	62.9-75.2	7
ACHARYA AB et al, 2011	105 (52M/53F)		all teeth (excluding M3)	Casts	Odontometric	Indian	76-100	6
ACHARYA AB et al, 2011a	203 (103M/100F)	19-32	33,43	Casts	Odontometric	Indian	50.2-65.7	7
BHARTI A et al, 2011	200 (100M/100F)	19-27	32,33,42,43	Casts	Odontometric	Indian	34.5-72.5	8
SASSI C et al, 2012	112 (56M/56F)	21-60	33,43	Casts	Odontometric	Uruguayan	72.3-77	9
SHARMA P et al, 2013	200 (100M/100F)	12-21	26,27	Casts	Odontometric	Indian	63-66.5	7
ANGADI PV et al, 2013	669 (323M/346F)	18-32	all teeth (excluding M3)	Casts	Odontometric	Indian	68.1-73.9	10
SHANKAR S et al, 2013	183 (90B/93G)	5-13	53,54,55,63,64,65	Casts	Odontometric	Indian	87.2-88	9
KHAMIS FM et al, 2014	400 (200M/200F)	secondary school children	11,12,13,14,15,16,17,41,42,43,44,45,46,47	Casts	Odontometric	Malaysian	70.2-83.8	7
MITSEA A et al 2014	172 (64M/108F)	13.5-45	all teeth (excluding M2,M3)	Casts	Odontometric	Greek	72	7
MUJIB AB et al, 2014	100 (50M/50F)	17-25	13,16,23,26	Casts	Odontometric	Indian	71	7
GARGANO V et al, 2014	1000 casts (475 maxillary (237M/238F)/525 mandibular (264M/261F))	18-60	13,23,33,43	Casts	Odontometric	Uruguayan	45.9-50.52	8
SREEDHAR G et al, 2015	60 (30M/30F)	19-30	33,43	Casts	Odontometric	Indian	75.8-84.3	6
NARANG SR et al, 2015	410 (210M/210F)	20-40	16, 26, 36, 46	Casts	Odontometric	Indian	67.5-88	7
MANCHANDA AS et al, 2015	200 (100M/100F)	18-57	11,12,13,14,15,16,17,41,42,43,44,45,46,47	Casts	Odontometric	Indian	51-80	7
PECKMANN TR et al, 2016	303 (126M/177F)	13-37	11,12,13,21,22,23	Casts	Odontometric	Chilean	54.4-63.3	7
BABBU SS et al, 2016	132 (66M/66F)	15-25	all teeth (excluding M3)	Casts	Odontometric	Indian	88	6
LAGOS D et al, 2016	150 (65M/85F)	18-24	33,43	Casts	Odontometric	Brazilian	54.57-85.24	7

*M/F = males/females, FDI = Federation Dentaire International, QAS = Quality Assessment Score, I2 = lateral incisor, PM2 = second premolar, M2 = second molar,
M3 = third molar

Table 4 - Sex estimation based on odontometric measurements performed on skeletal remains

Study	Included Subjects	Age Range	Examined Teeth	Examined on	Method of measurement	Origin Population	Sex Estimation Accuracy	QAS
	Total, M/F*	**(years)**	**(FDI)**				**(%)**	
TESCHELER-NICOLA M, 1992	172 (85M/87F)		deciduous/permanent teeth	Skeletal	Odontometric	Austrian - Early Bronze Age	75-81	6
INTRONA F Jr et al, 1993	80 (40M/40F)	3-11	54,55,64,65	Skeletal	Odontometric	Italian	80	6
ALT KW et al, 1998	166			Skeletal	Odontometric	German	41	6
PATTENATI-SOUBAYROUX I et al, 2002	146		permanent teeth (1284maxillary/1432mandibular)	Skeletal	Odontometric	French	58	6
ZADZINSKA et al, 2008	113	children	all deciduous teeth	Skeletal	Odontometric	Polish	69-88	7
CARDOSO HF, 2010	46	0-10	all deciduous teeth	Skeletal	Odontometric	Portuguese	33.3-75	6
VICIANO J et al, 2011	117(52M/35F/30subadults)	4-60	deciduous/permanent teeth	Skeletal	Odontometric	Herculaneum (Naples, Italy) -Italian	76.5-100	9
HASSETT B, 2011	123	subadults/adults	13,23,33,43	Skeletal	Odontometric	British	93.8-95	10
ZORBA E et al, 2012	107 (53M/54F)	16-86	26,27,28,36,37,38	Skeletal	Odontometric	Modern Greek	75-93	7
ZORBA E et al, 2013	101 (51M/50F)	16-85	26,27,36,37	Skeletal	Odontometric	Modern Greek	65.5-88.4	7
VICIANO J et al, 2013	269 (150M/119F)	infants/young children/adults	deciduous/permanent teeth	Skeletal	Odontometric	Spanish	78.1-93.1	8
VICIANO J et al, 2015	149	0+	all permanent teeth	Skeletal	Odontometric	3 proto-historic populations - Italian	79.31	9
PECKMANN TR et al, 2015	103 (53M/50F)	16-66	26,27,28,36,37,38	Skeletal	Odontometric	African American	40-77.6	5

*M/F = males/females, FDI = Federation Dentaire International, QAS = Quality Assessment Score

Table 5 - Sex estimation based on measurements performed on radiologic imaging

Study	Included Subjects	Age Range	Examined Teeth	Examined on	Method of measurement	Origin Population	Sex Estimation Accuracy	QAS
	Total, M/F*	(years)	(FDI)				(%)	
TARDIVO D et al, 2011	58	14-74	canines (113)	CT scan	Pulp volume/ tooth volume ratio		100	7
DE ANGELIS D et al, 2015	87 (41M/47F)	15-83	33	CBCT	Dental and pulp chamber volumes	Italian	80.5	7
TARDIVO D et al, 2015	210		13,23,33,43	CT scan	Total volume of tooth		82.38-85.24	6
CAPITANEANU C et al, 2016	200 (100M/ 100F)	22-34	21,22,23,24,25,26,27,28,31,32,33,34,35,36,37,38	X-ray (OPG - digital)	Odontometric	Caucasian	68-80	10
PAKNAHAD M et al, 2016	124 (64M/ 60F)	4-6	55,85	X-ray (bitewing)	Odontometric	Iran	68	8

*M/F = males/females, FDI = Federation Dentaire International, QAS = Quality Assessment Score, CT = Computer Tomography, CBCT = Cone Beam Computer Tomography, OPG = Ortopantomography

Table 6 - Sex estimation based on intraoral /photographs measurements

Study	Included Subjects	Age Range	Examined Teeth	Examined on	Method of measurement	Origin Population	Sex Estimation Accuracy	QAS
	Total, M/F*	(years)	(FDI)				(%)	
MACALUSO PJ, 2010	235 (130M/ 105F)	12-78	26,27	Photographs - Digitaly	Odontometric	South African Black	58.3-73.6	8
KHANGURA RK ET AL, 2011	100 (50M/ 50F)	20-30	11,12,13,21,22,23	Intraoral	Odontometric	Indian	58-64	7
MACALUSO PJ, 2011	235 (130M/ 105F)	12-78	26,27	Photographs - Digitaly	Odontometric	South African Black	59.6-74.5	9
KIRAN CS et al, 2015	120 (60M/ 60F)	15-40	33	Intraoral	Odontometric	Indian	72.5	8

*M/F = males/females, FDI = Federation Dentaire International, QAS = Quality Assessment Score

Table 7 - Cascade/Combinations of sex estimation methods

Study	Included Subjects Total, M/F*	Age Range (years)	Examined Teeth (FDI)	Examined on	Method of measurement	Origin Population	Sex Estimation Accuracy mean (%)	QAS
KAPILA R et al, 2011	40 (20M, 20F)	19-24	33,43	Intraoral, Casts, X-ray (periapical)	Odontometric		90	5
THAPAR R et al, 2012	200 (96M/ 104F)	18-30	11,12,13,14,15,16,17, 41,42,43,44,45,46, 47	Cranium, Intraoral or Casts	Cephalometric	Indian	70.95	6
PARAMKUSAM G et al, 2014	120 (60M/ 60F)	18-25	13,23,33,43	Intraoral, Casts	Odontometric	Indian	78.3	8
NADENDLA LK et al, 2016	120 (60M/ 60F)	20-30	33	Intraoral, X-ray (periapical)	Odontometric	Indian	74.5	8
RAJARATHNAM BN et al, 2016	200 (100M/ 100F)	18-25	33,43	Intraoral, Casts	Odontometric	Indian	73	7

*M/F = males/females, FDI = Federation Dentaire International, QAS = Quality Assessment Score

Table 8 - Sex estimation based on non-metric features of teeth

Study	Included Subjects Total, M/F*	Age Range (years)	Examined Teeth (FDI)	Examined on	Method of measurement	Origin Population	Sex Estimation Accuracy (%)	QAS
HUNT EE et al, 1955	93 (48B/45G)	2-8	16, 26, 36, 46	X-ray (lateral jaw, hand wrist)	Osseous and dental maturation	American Whites	73-81	4
ADLER CJ et al, 2010	151	children	53,54,55,63,64 ,65,73,74,75,83 ,84,85	Casts	Carabelli trait/ Molar cusp number	European derived Australian	70.2-74.8	6
HORVATH SD et al, 2012	120 (60M/ 60F)	19-29	11,12,13	3D-Casts	Crown shape analisis	Caucasian	53-65	7
RADLANSKI RJ et al, 2012	50	7-75	front tooth region	Intraoral photographs	Visual assessment front teeth		31-76	6

*M/F = males/females, FDI = Federation Dentaire International, QAS = Quality Assessment Score

Table 9 - Sex estimation methods based on biochemical analysis, cephalometric and sinuses measurements, cheiloscopy, and palatal features

	Study	Included Subjects	Age Range	Examined Teeth	Origin Population	Sex Estimation Accuracy	QAS
		Total, M/F*	(years)	(FDI)		(%)	
Biochemical	PILLAY U et al, 1997	45 (21M/24F)		M3		100	7
	KOMURO et al, 1998	20 (10M/10F)				100	4
	URBANI C et al, 1999	94 (50M/44F)				100	6
	MURAKAMI H et al, 2000	129 (64M/65F)				83.33-100	4
	SIVAGAMI AV et al, 2000	24	10-85	18,17,11,23,24,31,33,34,46	Indian	100	5
	VEERARAGHAVAN G et al, 2010	60 (30M/30F)	18-74	maxillary and mandibular PM/M	Indian	65-100	7
	SUAZO GI et al, 2010	40 (20M, 20F)	24-45	PM/M		100	5
	SUAZO GI et al, 2011	50 (25M/25F)	14-44		Chilean	100	7
	ZAPICO SC et al, 2013	14				100	5
	KHORATE MM et al, 2014	100 (50M/50F)				100	6
	ZAGGA AD et al, 2014	9		deciduous (I)/ permanent teeth(C,PM,M)	Nigerian Black/White	100	6
	SANDOVAL C et al, 2014	56 (28M/28F)	15-45	PM/M3		98.9	5
	KHANNA KS, 2015	90 (45M/45F)		PM/M	Indian	100	7

*M/F = males/females, FDI = Federation Dentaire International, QAS = Quality Assessment Score, I = incisors, C = canines, PM = premolars, M = molars,
M3 = third molar

Table 9 - (Continued)

	Study	Included Subjects	Age Range	Examined	Origin Population	Sex Estimation Accuracy	QAS
		Total, M/F*	(years)			mean (%)	
Cephalometric	SUAZO GIC et al, 2008	98 (63M/35F)	mean 39.3	Skull	Brazilian	75.50%	8
	SUAZO GIC et al, 2008a	108 (80M/108F)	mean 21.13	Mandible	Chilean	55.75%	9
	KONIGSBERG LW et al, 2009	3167		Skull	30 populations	57.91%	7
	OETLLE AC et al, 2009	653 (450 Black(396M/54F); 203 White(129M/82F))	21-98	Mandible	South African Black/White	61.80%	9
	GREEN H et al, 2009	144 (89M/55F)		Skull	Southeast Asian	86.80%	6
	NAIKMASUR VG et al, 2009	105 (55M/50F)	25-54	Skull	South Indian/ Tibetan	84.85%	7
	INDIRA AP et al, 2012	100 (50M/50F)	20-50	Mandible	Indian	76%	7
	CHANDRA A et al, 2013	100	18-62	Mandible	Indian	95%	6
	POONGODI V et al, 2015	200 (113M/87F)	4-75	Mandible	Indian	80.20%	6
	BADRAN DH et al, 2015	419 (126M/293F)	13-26	Mandible	Jordanian	70.90%	7
	DAMERA A et al, 2016	80	20-50	Mandible	Indian	83.80%	9
	SAMATHA K et al, 2016	120 (60M/60F)	18-45	Mandible	Indian	56.50%	8
	DEVANG DIVAKAR D et al, 2016	616 (380M/236F)	6.5-18	Skull	Indian	100%	7

	Study	Included Subjects	Age Range	Examined	Origin Population	Sex Estimation Accuracy	QAS
		Total, M/F	(years)			mean (%)	
Sinuses	TEKE HY et al, 2007	127 (65M/62F)	20-50	Maxillary sinuses	Turkish	69.3	6
	GOYAL M et al, 2013	100 (50M/50F)	21-54	Frontal sinuses	Indian	60	7
	BELALDAVAR et al, 2014	288 (147M/141F)	18-30	Frontal sinuses	Indian	64.6	7
	VERMA S et al, 2014	100 (50M/50F)	20+	Frontal sinuses		55.2	8
	PAKNAHAD M et al, 2016a	100 (50M/50F)		Maxillary sinuses	Iran	76	4

*M/F = males/females, FDI = Federation Dentaire International, QAS = Quality Assessment Score, I = incisors, C = canines, PM = premolars, M = molars,
M3 = third molar

Table 9 - (Continued)

	Study	Included Subjects	Age Range	Examined	Origin Population	Sex Estimation Accuracy	QAS
		Total, M/F	(years)			mean (%)	
Cheilioscopy	RANDHAWA K et al, 2011	600 (289M/311F)	1+	Lips	Indian	67.33	6
	SHARMA V et al, 2014	200 (100M/100F)	17-26	Lips	Indian	81	7
	NAGALAXMI V et al, 2014	60 (30M/30F)	20-30	Lips	Indian	85.05	7
	KAUL R et al, 2015	755 (375M/380F)	1-80	Lips	Indian	52.6	7
	Study	**Included Subjects**	**Age Range**	**Examined**	**Origin Population**	**Sex Estimation Accuracy**	**QAS**
		Total, M/F	**(years)**			**mean (%)**	
Palatal Features	BURRIS BG et al, 1998	332		Palate	American Whites/ Blacks	48	6
	SARAF A et al, 2011	120 (60M/60F)	22-26	Palatal Rugae	Indian	99.15	8
	BHARATH ST et al, 2011	100 (50M/50F)	15-30	Palatal Rugae	Indian	75.54	7

Table 10 - Accuracy of sex estimation methods meta-analysis

Subgroup	N	total RR FE (95% CI)	I^2	RE (95% CI)	I^2
measurements on casts	34	0.72 (0.71, 0.73)	95%	0.74 (0.70, 0.78)	95%
measurements on skeletal remains	13	0.75 (0.73, 0.77)	95%	0.73 (0.66, 0.82)	95%
radiologic imaging	5	0.79 (0.76, 0.82)	98%	0.81 (0.65, 1.01)	98%
intraoral/photography measurements	4	0.67 (0.63, 0.71)	13%	0.67 (0.64, 0.71)	13%
non-metric methods	4	0.68 (0.63, 0.72)	78%	0.67 (0.58, 0.77)	78%
cephalometric measurements	13	0.67 (0.66, 0.68)	100%	0.75 (0.31, 1.81)	100%
sinuses measurements	5	0.65 (0.62, 0.69)	69%	0.66 (0.59, 0.72)	69%
cheiloscopy	4	0.63 (0.61, 0.65)	97%	0.70 (0.56, 0.88)	97%
palatal features	3	0.64 (0.60, 0.68)	100%	0.71 (0.21, 2.47)	100%
biochemical methods	13	0.98 (0.97, 1.00)	51%	1.00 (0.98, 1.01)	51%
cascade/combination of methods	5	0.75 (0.71, 0.78)	70%	0.77 (0.71, 0.83)	70%

RESULTS

The established search string detected 4720 studies. 103 were considered eligible for inclusion after review of title / abstract and full-text (Figure 4).

There was a big range and variation between studies in terms of geographical and ethnic origin: Indian n=38 (36.9%), European n=17 (16.5%), Asian n=8 (7.8%), South American n=8 (7.8%), Middle East n=8 (7.8%), North American n=5 (4.8%), African n=4 (3.9%), mixed n=2 (1.9%), and unknown n=13 (12.6%).

Sample size varied between 9[31] and 755[19] subjects. Age range covered children (n=10; 9.7%), subadults (n=6; 5.8%) and adults (n=9; 8.8%), with several studies performed on more than one age group (n=58; 56.3%); age was not specified in 20 (19.4%) studies.

Sex estimation methods were classified based on dental metric and non-metric measurements (n=65), cephalometric analysis (n=13), frontal and maxillary sinuses (n=5), cheiloscopy (n=4), palatal features (n=3) and biochemical analyses of tooth materials (n=13).

Dental metric methods were mostly based on linear measurements of teeth (e.g. mesiodistal (MD) and/or buccolingual (BL) diameter), but also on intercanine distance), few studies performed volumetric measurements of teeth. Dental non-metric methods analysed crown shape, Carabelli's trait and molar cusp number. Cephalometric studies included linear and angular measurements. Methods based on sinuses performed linear and volumetric measurements. Cheiloscopy was based on lip print analysis, and palatal features like shape and rugae evaluation were considered for palatal methods. Biochemical analysis of teeth included DNA and Barr bodies.

Tooth measurements for sex estimation were mainly performed on casts (n=34), followed by skeletal remains (n=13), radiologic imaging (n=5), intraoral measurements/photography (n=4), and cascade of 2 of the above (n=4).

Non-metric dental features were analysed on dental casts (n=2), intraoral photography (n=1), and imaging (n=1). Cephalometric studies were performed on dry skull/mandible (n=4) and radiologic imaging (n=9). Linear and volumetric measurements of sinuses were done on radiologic imaging (X-ray/Computed Tomography/Cone Beam Computed Tomography). Palatal features were evaluated on casts.

Calculation of accuracy was based mainly on discriminant analysis. Linear regression analysis (LRA), principal component analysis (PCA) and area under the receiver operating characteristic (ROC) curve were other statistical methods used to determine accuracy.

Measurements on casts revealed an accuracy in sex estimation between 34.5%[32] and 100%[33] (Table 3). Measurements on skeletal remains were the object of fewer studies and had an accuracy of 33.3%[34] to 100%[35] (Table 4). For radiologic imaging accuracy ranged between 68%[36] and 100%[37] (Table 5). Intraoral/photography measurements gave an accuracy of 58%[6] to 74.5%[38] (Table 6).Use of cascade of 2 of the above methods did not increase accuracy in sex estimation, compared to individual methods, with a range between 70.95%[29] and 90%[39] (Table 7).

Non-metric methods had an accuracy between 31%[3] and 81%[40] (Table 8).

Cephalometric analysis resulted in accuracy range between 55.75%[41] and 86.8%[42] (Table 9). An accuracy of 55.2%[23] to 76%[24] was obtained for measurements on frontal maxillary sinuses (Table 9).

Cheiloscopy as a sex estimation method had an accuracy of 52.6%[19] to 85%[18] (Table 9). For palatal features the studies showed an accuracy of 48%[20] to 99.15%[21] (Table 9).

Accuracy of sex estimation reached 100% in most of the studies based on DNA analysis (Table 9). Combination of methods was used in one single study and showed increase of accuracy from 77.9% to 88.4%[29] (Table 7).

Quality assessment score ranged between 3 and 10 out of a maximum of 11 (Table 3-9). Only 4 studies (2.9%) reached the score 10, each of them had a different missing point, (related to ethical clearance, validation, discussion of analytical bias or selection bias).

Meta-analysis revealed the highest total RR for biochemical methods (Table 10) in both FE and RE models. In contrast cheiloscopy had the lowest total RR for the FE model, and sinuses measurements in the RE model (Table 10).

The highest weight result within the biochemical methods subgroup (n=13) was found in Murakami et al. (17.6% for FE model; 17.2% for RE model).

Heterogeneity was low for intraoral/photography measurements (I^2=13%) and biochemical methods (I^2=51%) (Table 10), compared to the highest of 100% for cephalometric measurements and palatal features (Table 10).

DISCUSSION

Multiple attempts have been made over the last over 60 years (since 1955)[40] to find a reliable sex estimation method to be used in dental identification. In our opinion reliable can be defined as showing an accuracy of over 80%, which can allow restricting in first instance the AM search to only the estimated sex. Because 80% is not providing an absolute discrimination, in forensic practice negative results in the first search, should be followed by a second search for AM data in both sexes. This is the first systematic review to illustrate in detail the different odontological sex estimation methods, and to compare their accuracy.

The majority of studies were performed on Indian population (36.9%), maybe due to a particular interest for measurements in these research groups.

The range of sample sizes was very wide (9 to 755), and the smaller the study group, the less reliable the statistical analysis results[43]. All studies had an equal distribution between males (M) and females (F), in order to avoid sampling bias.

The majority of studies chose young adult population as age range, to ensure that the teeth had the highest probability to be intact in case of dental measurements, and to have a complete skeletal bone development, not morphologically changed, in case of cephalometric methods. Dental sex estimation is used to narrow the search in forensic context and has to be applicable in all age groups, hence several studies were based on paediatric population only or included children and subadults/adults together. Outcomes from studies performed in young population could be extrapolated to adult population as a practical consequence, particularly in methods based on tooth length measurements. In this age group the probability to have intact mature dentition, not affected by attrition, is the highest[13].

Dental measurements on casts have been performed as early as 1978[44], and continued to be one of the most explored dental sex estimation method to date[45]. It represents the most used method based on teeth measurements, being easy to perform, largely available, inexpensive and suitable for retrospective data collection. In forensic context these measurements can be performed directly on recovered teeth.

According to this review tooth measurements on skeletal remains have been attempted later, in the early 90's[46], and most of the studies have been performed in the last 6 years[15, 16, 34, 47]. The lower number of studies is related to the fact that it is difficult to collect a large number of intact subjects with all deciduous/permanent teeth available in skeletal remains/corpses. Few studies based on radiological imaging were performed between 2011[37] and 2016[13, 36], some of them combining radiological measurements with intraoral/cast measurements[39, 48]. The low number likely results from the fact that radiographs mainly contain images of pathologic teeth (periapical X ray). Possible radiographic deformation, geometrical radiographic settings and image resizing according to the technical specifications of the unit manufacturer have to be considered[13]. Results obtained on panoramic/bitewing radiographs should be extrapolated as future research on periapical X ray, which represents the standard radiographic procedure in forensic practice. Along with the development of CT scans, tooth/pulp volumes were studied with regards to the ability to estimate the sex[37, 49]. Intraoral teeth measurements and intraoral photography represent also a more recent approach[6, 38], but direct measurements on teeth are technically more elaborate and require patient's compliance, and intraoral photography has to be taken with specific settings and magnification ratio to allow for accurate measurements. On the other hand photography is an essential part of dental post mortem documentation, so finding a reliable sex estimation method based on digital measurements performed on photographs would be helpful.

Cephalometric studies were mainly performed on mandibular medical imaging, including linear and angular measurements on panoramic radiographs[50, 51]. With increasing availability of CT scans, and more recently CBCT, measurements of frontal and maxillary sinuses were analysed for their potential of sex estimation[23, 24, 52]. The use of manual, semi-automatic or automatic software for segmentation can influence the outcome, as well as manual or automatic sinus(es) volume(s) calculation.

According to the present review cheiloscopy was considered as a method for sex estimation since 2011[18, 19], exclusively in studies performed on Indian population. One of the major disadvantages is that it cannot be applied in any stage of decomposition in corpses, limiting its use for early examination post-mortem. Similarly sex estimation based on palatal features has as main limitation the fact that it is not applicable in decomposed bodies, and was the subject of a low number of studies [20, 21].

Biochemical analysis of teeth started to develop in the late 90's [53], and is based on DNA and Barr bodies performed mainly on dental pulp tissue[54, 55]. DNA-Polymerase Chain Reaction (PCR) is expensive and requires a longer time to obtain the results, leading to an extended forensic identification process. In contrast, Barr bodies involve a quicker, less sophisticated technique and little equipment.

There was a wide range of accuracy in estimating sex for all these methods, between 33.3%[34] and 100%[31, 55]. Comparison of accuracies was difficult taking into account the variability of methods, populations and sample size, and also age range. However, biochemical methods, as expected, provided the highest accuracy, reaching 100% in most of the studies. DNA-PCR resulted in accuracy of 100%, except for one study reporting a decrease in accuracy with exposure of the dental pulp to high temperatures[54, 56]. Examination with fluorescent microscope only provided correct identification of sex in freshly extracted teeth[58]. Barr body analysis proved to have 100% accuracy in all studies except one[57], which reported an overall accuracy of 98.9%. Isolated accuracies of 100% were also reported in teeth measurements on casts[33], skeletal remains[35] and pulp/tooth volume ratios on CT[37], but haven't been reproduced in other similar studies. Two of the above studies[41, 43] also performed validation, but all authors described limitations of the methods, despite high accuracy. Acharya et al.[33] reported optimal sex prediction only when all teeth in both jaws were included, using logistic regression analysis. Viciano et al.[35] reported accuracy of 100% only for some of the canine dimensions used, and acknowledged inflation of accuracy due to small samples. Tardivo et al.[37] performed a preliminary study only, on a small sample size. However, as all the above authors concluded, these results must be interpreted with caution, and these methods cannot be used as solely sex estimation tool. It would be unrealistic in forensic context to avail of all teeth present.

Cascade of methods (e.g. intraoral + cast measurements, intraoral + radiologic measurements) were maybe expected to increase accuracy, but the reported range between 70.95-90% is similar to individual methods. Maybe this can be explained by the low number of studies to date (n=8), the use of only 2 different methods, and analysis of a limited number of teeth. Accuracy was shown to increase by using a combination of parameters (cephalometric and odontometric), compared to individual accuracies for each method in one single study[29].

Although all studies had clearly defined research questions, parameters and outcomes, lack of inclusion/exclusion criteria, validation, reliability tests and discussion of bias accounted for low score in most of them. This indicates that most authors did not follow a strict scientific research protocol.

Meta-analysis reinforced that biochemical methods were the most accurate compared to the others, in both FE and RE models. Biochemical analyses had the highest RR of 0.98 and 1.00 for FE model and RE model respectively. This is explained by the scientific precision of the method, due to automated registration/processing of available evidence, and to the fact that biochemical predictors (e.g. DNA) are 100% discriminative. In contrast, other methods were based on predictors with lower discriminative value, and more subjected to human error during data registration.

The slight increase of total RR when using the RE model versus FE model is due to the fact that RE model takes also into account studies unpublished or to be undertaken in the future. In view of the fact that the meta-analysis showed a high heterogeneity between studies ($I^2 > 75\%$ in the majority of the methods analysed n=7/11, 64%), the results of the RE model should be considered. Also the relative weights assigned under RE should be more balanced than those assigned under FE[58]. Changing from FE to RE, large studies will lose influence (e.g. Figure 5, Gargano et al. 2014[59]), and small studies can gain influence (e.g. Figure 5, Karaman, 2006[60]). RE model is justified if the analysed studies are not functionally equivalent[58]. It also allows extrapolating the outcome to other populations, which is the practical goal in forensic context.

Similar sample size and high precision reflected in overlapping CIs accounted for low heterogeneity in the intraoral/photography subgroup. In contrast, high variability in sample size and less overlapping of CIs was observed in the cephalometric and palatal studies, resulting in a high heterogeneity (Table 10).

CONCLUSION

The variety and high number of published odontological sex estimation methods highlighted the need and importance of sex estimation in human identification.

Biochemical analysis of teeth was the most accurate odontological sex estimation method, with accuracies ranging between 82.5 and 100%, but it has limitations in forensic practice. The restrictions are related to the necessity of having biochemical predictors with high quality and quantity in the available odontological evidence and the costs and time needed to perform the required analyses.

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Prevalence of agenesis of frontal sinus in human skulls with metopism

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ABSTRACT

Background: The frontal bone is an anatomical structure of the skull separated by the metopic suture in the childhood. The scientific literature indicates that metopic suture consolidates with closure in the early stages of life. Metopism is the term used to describe a metopic suture that persists up to the adulthood. Persistent metopic suture is associated potentially with the agenesis of the frontal sinus.

AIM: To investigate the prevalence of absent frontal sinuses in dry skulls with metopism.

Materials and methods: The present study was performed after the approval of the local Committee of Ethics in Research. The sample consisted of dry skulls (n=245), aging between 17 and 50 years old, of the Forensic Medical Institute of Goiânia, Brazil. The skulls underwent anthropological exam in the search for metopism. Radiographic exam was performed in the skulls with metopism to verify the presence or absence of the frontal sinus. The radiographic assessment was performed with a Mobile DaRt Evolution device (Shimadzu, Kyoto, Japan) with protocol set in 64 kV and 16 mA).

Results: From the 245 dry skulls, 17 presented metopism. The length of the metopic suture in the skulls, considering the distances between nasio and bregma craniometric landmarks, ranged between 114 mm and 137 mm. Radiographic exams were performed on 16 skulls (one skull was not analysed radiographically because of extensive destruction). Only one skull (6.25%) had the frontal sinus absent. Besides the agenesis, the present study also found four (12.5%) skulls with aplasia and eight (25.0%) hyperplasia of the frontal sinus in dry skulls with metopism.

Conclusion: The present study found a low prevalence rate of the agenesis of frontal sinuses in dry skulls with metopism.

INTRODUCTION

Sutures are fibrous joints present in the skull since foetal age. They develop from the final stage of the fontanelles until the adulthood. In the adulthood most sutures tend to disappear in a process called synostosis, which consists of the replacement of fibrous tissue by bone tissue.[1]

One of the exceptions for sutures closure, in terms of age, refers to the frontal bone that is even in individuals under two years age and is separated by the metopic suture (or frontal suture).[2] From this age group and in some later cases (up to 7 years) this suture tends to disappear.[3, 4]

While in one hand the disappearance of the metopic suture is a natural process, in the other hand this suture may persist in the adult.. This anatomical condition is known as metopism or persistent metopic suture.[2, 5]
The frontal bone is described as pneumatic because it has a cavity in its interior called the frontal sinus.[6, 7] This cavity is not radiologically evident in the first years of life. During childhood the development of the frontal sinus is influenced by the osteoclastic activity in the region of the ethmoidal cells.[8, 9]
The development of the frontal sinus bilaterally is independent. Yet the morphology of these sinuses is distinctive for each individual.[6] After full maturation, which occurs in adolescence or early adulthood, the contour and expansion of the frontal sinus becomes practically immutable.[10, 11]
Based on the fact that the radiographic morphology of the frontal sinus is highly distinctive, it becomes very useful for human identification in complex cases, such as those involving carbonized and putrefied bodies, as well skeletal remains.[12]
Moreover, studies point to persistent metopic suture as a factor that could interfere in the development of the frontal sinus uni- or bilaterally, leaving it atrophic or absent.[5, 13, 14] Thus, the relevance of investigating the prevalence of the agenesis of frontal sinus within subjects with metopic suture relies on potential application for human identification in Forensic Odontology. More specifically, the agenesis of frontal sinuses represents a distinctive aspect to be considered when detected post-mortem. This finding could contribute significantly for human identifications if tracked back in ante-mortem data.
The aim of this study was to investigate the prevalence of absent frontal sinuses in dry human skulls with metopism.

MATERIALS AND METHODS

This study was approved by the Committee of Ethics in Research from the Federal University of Goiás (CAAE: 55409816.9.0000.5083).
At first, all the skeleton filed at the Forensic Medical Institute of Goiânia until the end of July 2016 were examined to visually identify the presence of the persistent metopic suture in adult skulls. Incomplete skeletons in which the skulls were absent or fragmented were excluded from the sample and the skulls of children under 5 years old. Skulls with the region of the frontal bone adjacent to the ceiling of the orbits fragmented, making impossible the accomplishment of radiographic images, were also excluded from the sample.
Once the metopism was identified, the anthropological examination of the skeleton was performed by means of visual examination, which allowed the assessment of sex. This procedure was performed following the standards of Buikstra and Ubelarker (1994)[15] considering four qualitative (morphological) sexual parameters in the skull, one in the mandible and one in the pelvic bones. The length of the metopic suture was also assessed, as well as the cause of death. In this step all the skulls were photographed (Figure 1) together with their respective mandibles. The age of the skeletal remains was assessed following the recommendations of Mckern and Stewart (1957)[16] and Watanabe and Terazawa (2006)[17], which consider the progressive alterations in human bones adulthood, such as the development of osteophytes.
Within the research sample, the skulls that presented metopic suture, intact frontal bone and with the possibility of radiographic analysis, were submitted to post-anterior skull radiography. This radiographic examination was performed the MobileDaRt Evolution device (Shimadzu, Kyoto, Japan), having exposure of 64 kV and 16 mA.
After the skull radiographic acquisition, the images were interpreted based on the presence or absence of the frontal sinus, as well as its size. Adobe Photoshop CS4® software was used to visualize and evaluate frontal sinus characteristics.
As a criterion to determine the frontal sinus size in the individuals of the sample, the study of Ribeiro (2000)[18] was used as reference. In the study, the author reported a method of measuring the frontal sinus on radiographs, using measures of maximum height and maximum width of the cavity examined. If a skull does not have frontal sinus, only the distance between the projection of the lines passing through the median edges of the orbital cavities is measured (X).
To aid in the determination of frontal sinus size, the study described by Guerram et al. (2014)[19] was also used. The study applied a classification method to determine differences in frontal sinus development patterns in individuals with and without metopism from radiographs. Using two perpendicular lines (one line on the supraorbital

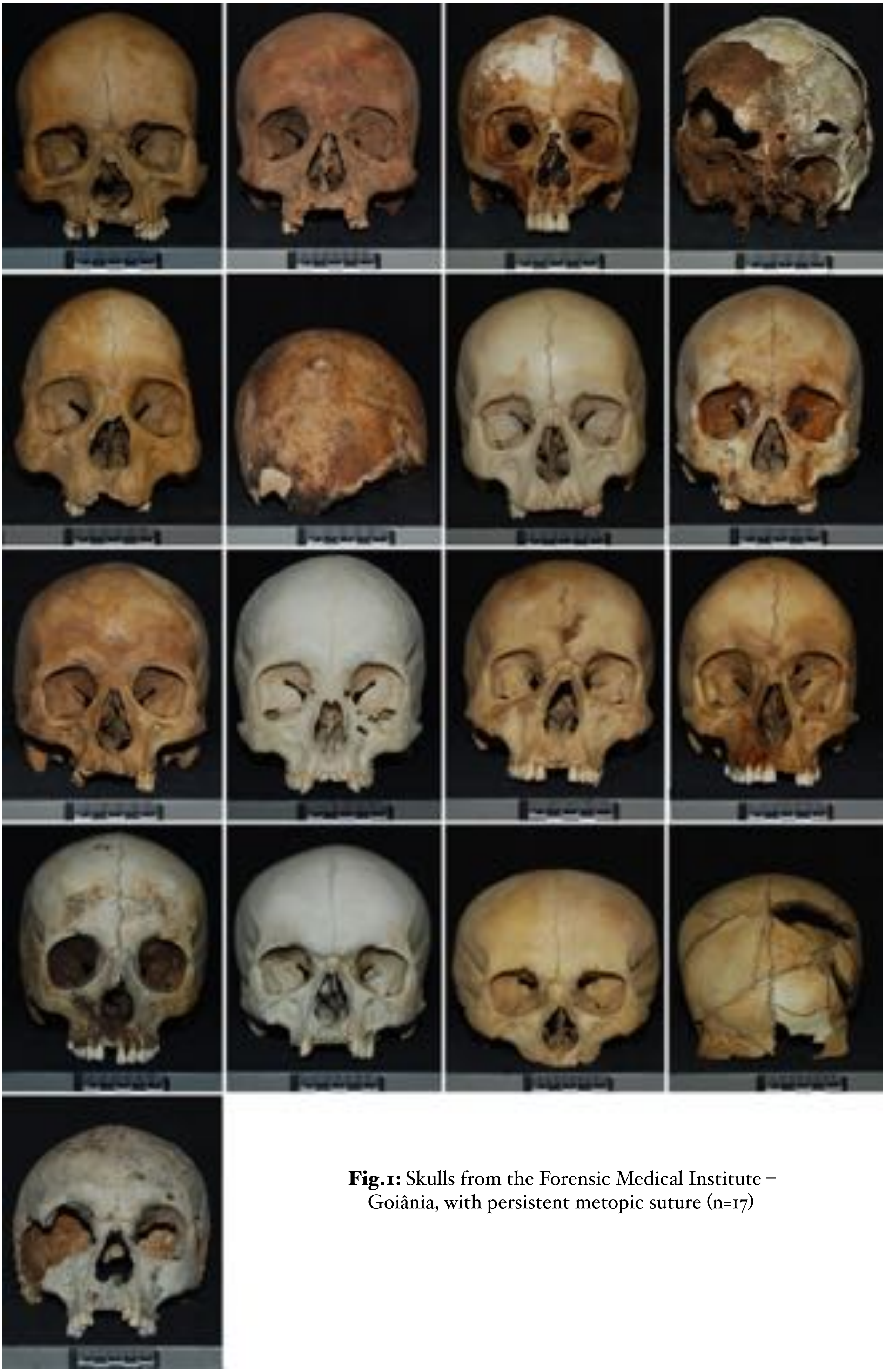

Fig.1: Skulls from the Forensic Medical Institute – Goiânia, with persistent metopic suture (n=17)

margin, the other drawn in the middle of the orbital cavity), Guerram et al. (2014)[19] classified frontal sinus size into four categories: (1) aplasia: absence of the frontal sinus; (2) hypoplasia: frontal sinus limited to the supraorbital line area; (3) medium size: frontal sinus limited to the medial area of a line drawn in the middle of the orbital cavity; and (4) hyperplasia: frontal sinus that extends in the lateral area to the line drawn in the middle of the orbital cavity.
The data obtained from the examination of the skull radiographs (frontal sinus presence or not) and the anthropological examination (sex, estimated age, presence of the metopic suture and frontal sinus size) were tabulated in Microsoft Office EXCEL 2016 (Microsoft, USA). The SPSS (Statistical Package for Social Sciences, USA) version 21 was used for statistical analysis.

RESULTS

In a total of 266 skeletons examined at the Forensic Medical Institute of Goiânia, 245 had a skull. Of the skulls evaluated, 17 presented the persistent metopic suture (6.94%). Among these

Fig.2: Photographs of skulls with metopism and their respective radiograph.

(A) Skull with frontal sinus (B) Skull with absent frontal sinus.

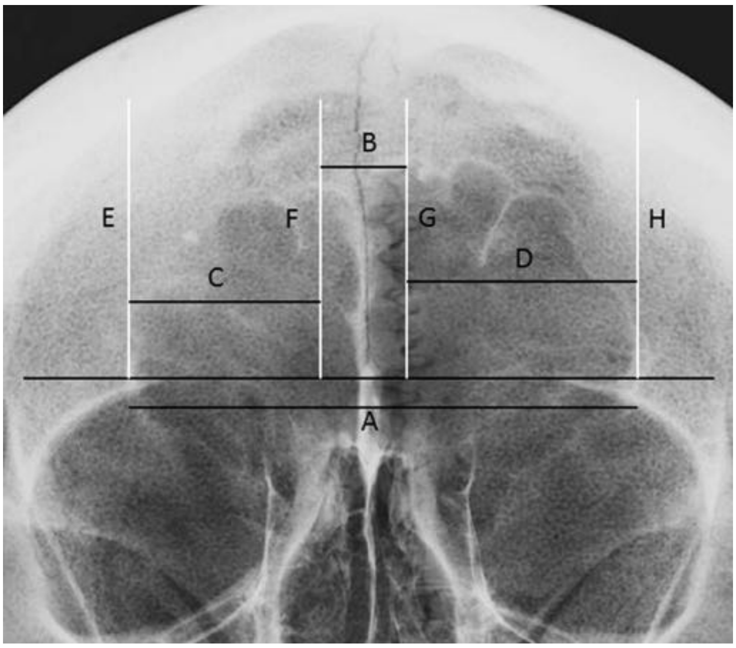

Fig.3: Parameters of Ribeiro (2000) [18] to analyse the frontal sinus: a baseline is drawn horizontally along the upper limit of both orbital cavities. Four lines are then drawn perpendicular to the baseline. One line E delineates the maximum lateral limit of the right frontal sinus. Another line F passes through the highest point of the right frontal sinus. The line G is drawn through the highest point of the left frontal sinus and the line H defines the most lateral limit of the left frontal sinus. Measurement A is the maximum width of the frontal sinus. Measurement B is the distance between F and G lines. Measurements C and D are the distance between E to F lines and G to H lines, respectively.

skulls, with confirmation of the condition of metopism, a skull was excluded from the sample for evidencing an extensive fracture. From the 16 skulls that composed the radiographic sample with metopism, 6.25% presented complete absence of the frontal sinus.

The length of the metopic suture in the skulls, considering the distances between the nasal and bregma craniometric points, ranged from 114 mm to 137 mm. The estimated age varied between are located below the baseline they are negative (N); and when they are situated in the baseline, these points are classified as null (0). From the studied radiographs (n = 14) ten presented type P; one type N with P; one type N with 0; one type P with N and 0; and one skull revealed an absent frontal sinus (X = 1.36 cm).

Table 1 also describes data on sexual dimorphism. Three skulls were not included because they presented a fracture in calvaria. From the parameters chosen for analysis, using the skull and mandible, six (35.3%) of the cases indicated female gender, six (35.3%) suggested male gender and two (11.8%) were indeterminate. In relation to pelvis analysis for sexual dimorphism, it indicates one (5.8%) female, nine (53%) male and seven (41.2%) undetermined. The concordance between examiners considering sexual dimorphism ranged between 0.71-1.0, which suggest an almost perfect concordance (Table 2).

Table 3 shows differences in frontal sinus development patterns in the individuals of the sample who have metopism, using the same classification method that Guerram et al. (2014)[19] applied in their study. In addition to agenesis, the present study also found four (12.5%) skulls with aplasia and eight (25.0%) with frontal sinus hyperplasia.

DISCUSSION

The cranial sutures influence the development of the entire skull, act on the modelling of the head growing and also during childbirth. Premature fusions of these sutures may be responsible for several conflicts between the growth of the skull and the encephalus.[1] However, there is still no consensus on the correlation between frontal sinus development and late closure of the metopic suture. From the forensic scope, metopism should be considered an important tool for human identification. While in one hand the frontal sinus itself has a distinctive morphology, in the other hand the absence of subadult individuals, adults and elderly according to Table 1. It was observed in the radiographic examinations that the frontal sinus was present in 15 cases and agenesis was present in only one skull. Figure 2 illustrates these two conditions found.

According to the classification described by Ribeiro (2000)[18], when the points delineated in lines E, F, G and H are above the baseline, they are classified as positive (P) (Figure 3); when they frontal sinuses in adult skulls is uncommon and could be considered a distinctive identifier even more valuable if tracked back in ante-mortem data. Investigating the interrelationship between metopism and agenesis of frontal sinuses could contribute significantly in the routine of medicolegal institutes.

Persistent metastatic suture is an anatomical variation which has great clinical relevance since it can be confused with traumatic skull fractures, and can also be an important characteristic in human identification. This suture can be easily evidenced in anthropological analyses for forensic purposes, as well as in conventional radiographs when the calvaria is not exposed. An important feature that differs the persistent metopic suture from a fracture is its sclerotic borders seen on skull radiographs.[13, 20] Errors in this diagnosis can lead to misguided therapies and unnecessary interventions.[21]

Metopism occurs with a relatively low frequency. Berry and Berry (1967)[22] showed a worldwide incidence between 0-7.4% in individuals of different ethnic groups. Several countries show different results, and in the Brazilian population there are discussions about incidence. In studies at Brazil, Del Sol et al. (1989)[23] evaluated 400 skulls, showing an incidence of metopism of 2.75% (2.96% in women and 2.64% in men). Castilho et al. (2006)[24], in an evaluation of 71 skulls, reported an incidence of 7.04% (14.28% for women and 2.32% for men). Da Silva et al. (2013)[25] evaluated 134 skulls, of which 13 presented persistent metopic suture (61.5% were male, and 38.5% female), with 4.48% with complete persistent metopic suture (without sex predominance) and 5.22% with incomplete persistent metopic suture (mild predominance among men). In this present study it is noticed that the results differ, observing a prevalence of 6.25%.

In view of these disagreements and considering that the four studies analysed Brazilian skulls, it

Table 1 - Estimated age determined by anthropological evaluation and skulls metopic suture length, size and classification of the frontal sinus according to Ribeiro (2000)[18] and sexual dimorphism evaluation in the sample (cranium and pelvic analysis) (n=17)

SKULL NUMBER	ESTIMATED AGE (YEARS)	MS MEASURE (MM)	GENDER ACCORDING TO SKULL ANALYSIS	GENDER ACCORDING TO PELVIS ANALYSIS	A(CM)	B(CM)	C(CM)	D(CM)	TYPE
1	30-40	#	Impaired	Male	-	-	-	-	-
2	30-40	122	Male	Male	3,55	1	1,73	0,82	P
3	30-50	122	Female	Absent	3,49	1,61	1,19	0,69	P
4	30-40	#	Impaired	Male	-	-	-	-	-
5	30-40	#	Impaired	Male	-	-	-	-	-
6	> 50	134	Male	Fractured	4,04	1,04	1,36	1,64	P
7	30-40	137	Male	Male	3,4	1,04	1,09	1,27	P
8	> 40	125	Undefined	Absent	3,73	1,64	1	1,09	P
9	20-30	130	Female	Undefined	4	1,23	1,45	1,32	P
10	30-40	126	Female	Female	1,36	0	0,45	1	P,0
11	30-40	128	Female	Male	2,54	1,45	0,77	0,32	P,0
12	30-40	131	Male	Male	4,09	1,27	0.82	2	P
13	17-23	130	Female	Male	1,31	0	0,77	0,54	P,N,0
14	17-30	114	Undefined	Absent	-	-	-	-	X=1,36
15	20-30	131	Female	Absent	2,72	1,27	0,45	1	P
16	> 50	120	Male	Absent	2,64	0,82	0,91	0,91	P
17	> 50	121	Male	Male	4,17	1,54	1,45	1,18	P

P = positive; N = negative; 0 = null; X = distance between the orbits; A = the maximum width of the frontal sinus; B = distance between F and G lines; C = distance between E to F lines; D = distance between G to H lines.

Table 2: Intra and inter-examiner kappa values in the study of sexual dimorphism through the skull and mandible, according to Buikstra & Ubelaker (1994).[15]

TYPE OF COMPARISON	KAPPA VALUE	AGREEMENT
E1 D1 X E1 D2	1	Almost perfect
E2 D1 X E2 D2	0,81	Almost perfect
E1 D1 X E2 D1	0,80	Almost perfect
E1 D2 X E2 D2	0,71	Substantial

E = examiner; D = day.

Table 3: Frontal sinus classification according to its size in the skull that presented persistent metopic suture (n=28)

SINUS DEVELOPMENT	APLASIA (L)	HYPOPLASIA (L)	MIDIUM (L)	HYPERPLASIA (L)	Total
APLASIA (R)	1	0	2	0	3
HYPOPLASIA (R)	0	0	0	0	0
MIDIUM (R)	0	0	5	3	8
HYPERPLASIA (R)	0	0	1	2	3
Total	1	0	8	5	28

is believed that these data can be explained by the differences in the sample size, and also by the population difference that exists between the geographic regions of Brazil. In their study, Del Sol et al. (1989)[23] obtained the skulls of the south-eastern region of Brazil, while in the research developed by Castilho et al. (2006)[24] the skulls were from southern Brazil, and Da Silva et al. (2013)[25] searched for skulls in north-eastern Brazil. In the present study, skulls from the central-western region of Brazil were evaluated.

Considering the length of the complete metopic suture (present between the bregma and the nasal points), the present study showed an average length of 126.9 mm, which is slightly lower than that found by Castilho et al. (2006)[24], who described an average of 129.2 mm.

Some authors have reported that the presence of persistent metopic suture influences frontal sinus development.[5, 13, 14] In this study, the classic affirmation of this possible relationship was not confirmed.

Regarding frontal sinus size, it usually presents bilaterally, asymmetric, and has a median septum splitting the right and left sides.[25] This morphology remains practically unchanged during adulthood, although some factors can change this structure, either through hyperpneumatization from sports activities, diseases, infections, tumours or traumas, and other factors.[18]

Bilgin et al. (2013)[26] in a study evaluating persistent metopic suture and frontal sinus development, analysed 631 computed tomography and MRI images of skulls. The presence of atrophied frontal sinus was confirmed in cases (22.7%), 61 cases revealed persistent metopic suture (9.7%), and 15 (2.4%) had persistent metopic suture associated with the atrophied frontal sinus. Of these 15 cases, six were related to bilateral frontal sinus atrophy. This study did not present significant results in the relationship between dimorphism and frontal sinus atrophies with the presence of the persistent metopic suture.

The present study shows that 6.25% of the skulls with metopism present complete absence of the frontal sinus. Guerram et al. (2014)[19] studied the frontal sinuses of 143 dry skulls, dividing them into two groups: 63 with metopism and 80 without metopism. Frontal sinuses with hypoplasia are much more frequent in skulls with the presence of persistent metopic suture (50.8% vs. 9.4%). The frontal sinus aplasia was slightly superior in skulls with metopism (7.1 vs 2.5%). The medium size was the most predominant condition in skulls without metopic suture (76.2 vs 40.5%), followed by sinus hyperplasia (11.9 vs 1.6%). Differently from these results, the present study observed that the majority of skulls with persistent metopic suture have the medium pattern (57.14%), while 12.50% are frontal sinuses with aplasia.

CONCLUSION

The present study found a low prevalence of frontal sinus agenesis in dry skulls with metopism, not evidencing the relation of the presence of this condition with the absence of frontal sinus development.

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Extra-oral dental radiography for disaster victims using a flat panel X-ray detector and a hand-held X-ray generator

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KEYWORDS

disaster victim identification, extra-oral plain dental radiography, flat panel X-ray detector, lateral oblique radiograph, mouth opening, scattered X-rays

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ABSTRACT

Background: Forensic odontologists commonly incise the skin for post-mortem dental examinations when it is difficult to open the victim's mouth. However, it is prohibited by law to incise dead bodies without permission in Japan. Therefore, we attempted using extra-oral dental radiography, using a digital X-ray equipment with rechargeable batteries, to overcome this restriction.

Materials and methods: A phantom was placed in the prone position on a table, and three plain dental radiographs were used per case: "lateral oblique radiographs" for left and right posterior teeth and a "contact radiograph" for anterior teeth were taken using a flat panel X-ray detector and a hand-held X-ray generator. The resolving power of the images was measured by a resolution test chart, and the scattered X-ray dose was measured using an ionization chamber-type survey meter.

Results: The resolving power of the flat panel X-ray detector was 3.0 lp/mm, which was less than that of intra-oral dental methods, but the three extra-oral plain dental radiographs provided the overall dental information from outside of the mouth, and this approach was less time-consuming. In addition, the higher dose of scattered X-rays was laterally distributed, but the dose per case was much less than that of intra-oral dental radiographs.

Conclusion: Extra-oral plain dental radiography can be used for disaster victim identification by dental methods even when it is difficult to open the mouth. Portable and rechargeable devices, such as a flat panel X-ray detector and a hand-held X-ray generator, are convenient to bring and use anywhere, even at a disaster scene lacking electricity and water.

INTRODUCTION

Six years have passed since the Great East Japan Earthquake, and the identity of 69 victims remain unknown.[1] Kumagai[2] reported the post-mortem dental records of unknown disaster victims in Iwate prefecture three years after the Great East Japan Earthquake. Of these, 64.7% did not have post-mortem dental charts available, and 79.4% had neither oral photographs nor dental radiographs. She reported that one of the reasons for few and poor dental records was that many burnt bodies were included, which were fragile and where it was difficult to open the mouth.

Forensic odontologists commonly incise the skin when it is difficult to open the victim's mouth for post-mortem dental examinations.[3,4] However, in Japan, it is prohibited by law to incise dead bodies without a permit, and it is difficult to obtain a permit from a court at a disaster site, because the permit requires that the same procedures as for forensic autopsy should be followed. Most dentists at sites lacking electricity and water could not record oral findings, take oral photographs, or take dental radiographs of the victims using intra-oral methods without a permit.

To record some dental information without the need for a permit, forensic odontologists have developed devices, such as cameras for obtaining oral photographs[5-7] and mouth gags. After the earthquake, we attempted to perform dental radiography by extra-oral methods to overcome this restriction. We usually take panoramic radiographs under recumbence[8] and multi-detector computer tomography (MDCT) images[9-12] as extra-oral methods in our institution. However, these radiographs require large appliances, and it is difficult to apply them outside of specialized institutions. Therefore, we attempted to take "lateral oblique radiographs" for posterior teeth and "contact radiographs" for anterior teeth (hereafter collectively termed "plain dental radiographs") using portable devices,[13,14] i.e. analog film and an alternating-current(AC) powered hand-held X-ray generator. We found that all teeth could be covered by only three radiographs, which could be taken by a single examiner when the body is placed in the prone position. Additionally, these radiographs yielded clear images sufficient for comparison to the ante-mortem images, and lead to positive identification.[15]

In this study, we evaluated the use of extra-oral plain dental radiography by means of portable and rechargeable-type devices, i.e. a flat panel X-ray detector and a hand- held X-ray generator, with a view to using these in disaster sites, and examined the scattered X-rays to determine the safety of use.

MATERIALS AND METHODS

Materials for extra-oral plain dental radiographs

The extra-oral plain dental radiographs were taken with a hand-held X-ray generator: NOMAD™ (Aribex, Charlotte, NC, USA), and a flat panel X-ray detector (FPD): FUJIFILM DR CALNEO C mini wireless SQ 24 x 30 cm (Fujifilm Medical, Tokyo, Japan).The NOMAD™ has rechargeable batteries and backscatter radiation shielding, and the anode voltage(60 kV) and anode current(2.3mA) were fixed. CALNEO C is an indirect conversion type X-ray detector, and the images were immediately sent to a laptop with wireless.

Methods for extra-oral plain dental radiographs

The FPD and ahead phantom for dental radiographic examinations (Kyoto Kagaku, Kyoto, Japan)were placed in the prone position. We used three plain dental radiographs per case; we used "lateral oblique radiographs" for posterior teeth on both sides and a "contact radiograph" for anterior teeth.[14] For "lateral oblique radiographs", the FPD was placed against the cheek to cover the premolars and molars. The central X-ray was projected from between the ramus and the cervical spines of the contralateral side toward the posterior teeth (Figure 1A). For "contact radiographs", the FPD was placed against the lips to cover the incisors, and the central X-ray was projected from the posterior neck region toward the anterior teeth (Figure 1B). For anterior teeth, the cone position of the X-ray generator was placed closely against the neck skin to enlarge the cervical spine.

Measurements of the resolving powers

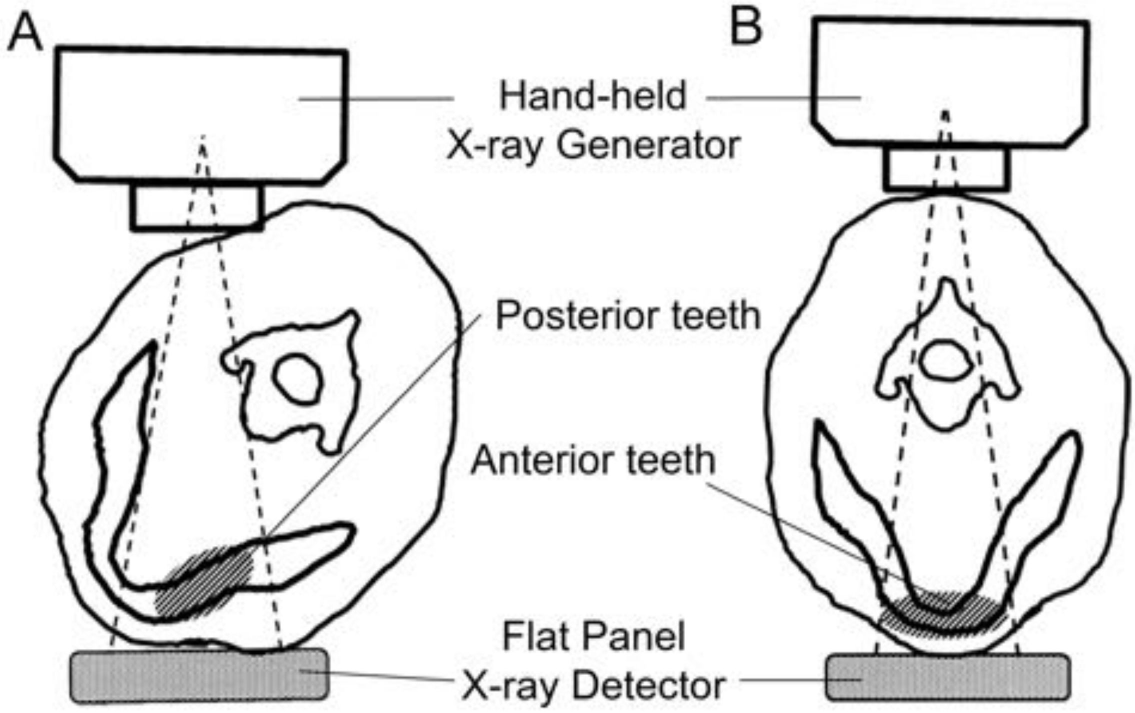
The resolving power of the images was measured using a resolution test chart type 1 (Moriyama X-ray Equipment, Tokyo, Japan), for paired use of the CALNEO C and NOMAD™. As a reference, we also measured the resolving power for paired use of a Compuray Ultimate Sensor Large (Yoshida, Tokyo, Japan), which is a Complementary Metal Oxide Semiconductor (CMOS)censor for intra-oral radiography, and a NOMAD™.

Measurements of the dose of scatted X-rays

The FPD and the head phantom were placed in the prone position on a 70-cm high table. The X-ray generator was set in the positions for taking images of the left posterior teeth, anterior teeth, and right posterior teeth, and irradiation was performed 6 times foro.5 second for each radiograph.

Fig.1: Extra-oral plain dental radiography.

(A) The "lateral oblique radiograph" method for the posterior teeth.
(B) The "contact radiograph" method for the anterior teeth.



An ionization chamber-type survey meter ICS-323C (Hitachi Aloca Medical, Tokyo, Japan) was placed on a 1 m high table, toward the cone of the X-ray generator. The scatted X-rays were measured[16,17] at distances ranging from 0.5 m to 2.0 m, at 0.5 m intervals from the cone of the X-ray generator, and at angles ranging from 0° to 180°, at 45° intervals with respect to the phantom's body-axis line. We set 0° as the direction to the feet, 90° as the right side of the phantom, and 180° as the direction to the top of the head. A bone model with a 2.5 mm thickness lead-equivalent apron stood was placed at 0.5 m, 180°, to represent an examiner. Therefore, the 1 cm dose equivalent was measured at 19 measurement points and the examiner standing point after removing the bone model.

Calculation of the dose of scatted X-rays

The dose equivalents of scatted X-rays for one case were calculated. One case involved three dental radiographs: left posterior teeth, anterior teeth, and right posterior teeth, and the exposure time for each radiograph was 0.10 s.

Furthermore, the average of the maximum number of cases of plain dental radiographs that could be taken in one day, while remaining within the individual dose limits,[18] i.e. 20 mSv/year for occupational exposure and 1 mSv/year for public exposure, were calculated, with one year equal to 50 weeks and one week equal to five days.

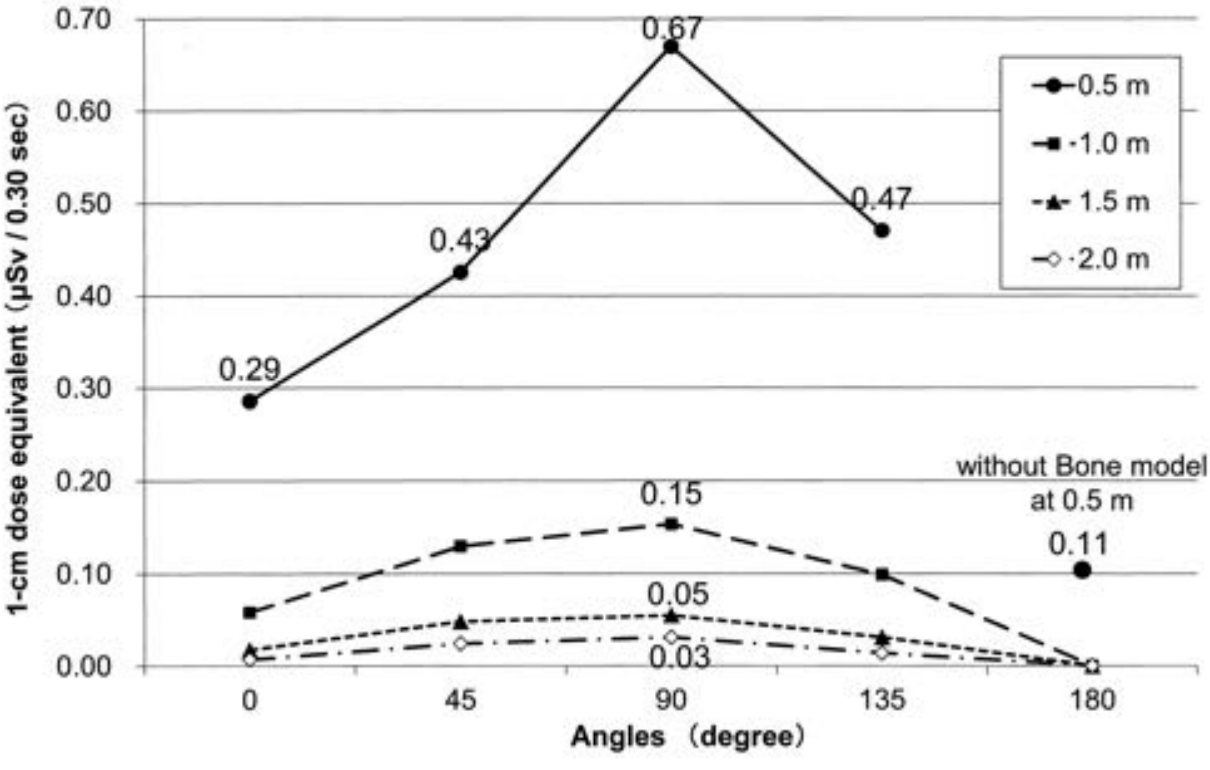
RESULTS

We attempted to take plain dental radiographs of a head phantom using the FPD and the rechargeable hand-held X-ray generator by the same way as using the analog films and the AC powered hand-held X-ray generator. Teeth were comprehensively covered using only three radiographs (Figure 2).When the exposure time was over 0.06 s, the images were good quality automatically by the image processing software.

The resolving powers of the digital devices were measured. The resolving power using the CALNEO C and NOMAD™ was 3.0 line pairs/ mm (lp/mm), and that by Compuray Ultimate

Fig.2: The three extra-oral plain dental radiographs of the head phantom. The three radiographs provided a broader coverage from outside of the mouth.

Fig.3: The 1 cm dose equivalents of scattered X-rays of one case, at various distances and angles. A higher dose was observed at 90° for each distance, and the dose decreased approximately with the square of the distance



Sensor Large and NOMAD™ was 6.0 lp/mm.

The average dose of scatted X-rays for one case was measured and is shown in Figure 3. The highest dose was 0.67 μSv/case at 0.5 m, 90°. The higher dose of scattered X-rays was observed at 90° for each distance, and the dose decreased approximately with the square of the distance. The dose at the measurement points behind the bone model were less than 0.1 μSv for 3-s exposure. The dose at the examiner's standing

point was 0.11 µSv/case.
The averages of the maximum number of cases of plain dental radiographs that could be taken in one day, while remaining within the individual dose limits, were calculated and are shown in Table 1. If examiners registered as radiation workers, they could process up to 119 cases per day without the need for protective devices. However, in view of public exposure, they could take up to 5 cases at 0.5 m, up to 26 cases at 1.0 m, and up to 80 cases at 1.5 m.

Table 1: The average of the maximum number of cases of plain dental radiographs that could be taken in one day, while remaining within the individual dose limits.

Distances (at a 90° direction)	Dose of Scattered X-rays of 1 case	Average of maximum number of cases in one day	
		Occupational exposure < 20 mSv / year	Public exposure < 1 mSv / year
0.5 m	0.67 µSv	119 cases	5 cases
1.0 m	0.15 µSv	533 cases	26 cases
1.5 m	0.05 µSv	1,600 cases	80 cases
2.0 m	0.03 µSv	2,666 cases	133 cases

DISCUSSION

Plain dental radiographs obtained by extra-oral methods using the FPD and the rechargeable hand-held X-ray generator recorded post-mortem dental information objectively equivalent to those using analog films and the AC powered hand-held X-ray generator. As compared to radiography using films, the following points were the same. Three radiographs were sufficient to cover all teeth, and were obtained by one examiner when the dead body was in the prone position. The overall dental information, such as teeth, alveolar bone, and the structures in the maxilla and mandible, could be recorded from outside of the mouth using this approach. Radiopaque dental restorations, severe dental caries, and radiolucent or radiopaque lesions in the jaws are also likely to be observable, because those could be seen in the study using films. Moreover, radiography using the FPD has advantages, because the FPD is a digital device. We were able to assess the images obtained immediately, without the need for film development; no water is needed for such development, and the image could be retaken if the original image was not adequate. The range of exposure time was not particularly rigorous, because we were able to use image-processing tools. No electricity is needed in mortuaries, because the equipment is rechargeable.
We measured the scattered X-rays when the plain dental radiographs were taken. Before taking measurements, we decided on the exposure time. The range of exposure time required for obtaining plain dental radiographs with the FPD is not narrower than that required for analog film. We were able to take one radiograph per 0.01 s of exposure time, but the image quality was low. In the preliminary test in practice, one radiograph with good quality could be obtained within 0.10 s for almost all cases, although the adequate exposure time depended on the condition of the dead body. We recorded the dose of the scattered X-ray per case as a 1-cm dose-equivalent for 0.10 s exposure time for each of three regions, totalling 0.30 s.
The highest dose of scattered X-rays was 0.67µSv /case at the point of 0.5 m and 90°. Takahashi[16] reported that the maximum dose of scattered X-rays was approximately 3 µSv, when 10 intra-oral radiographs were taken for all teeth. The dose for extra-oral plain dental radiographs

was markedly less than that for intra-oral dental radiographs, because the central X-ray was projected toward the floor. The dose of scattered X-rays decreased approximately with the square of the distance, and the dose was distributed higher at 90° and lower at 0° and 180°. This distribution was observed not only for the irradiation of the right molars, but also for the left molars, probably because the neck region was concave between the head and the body.

As the public are nervous about radiological protection after the nuclear accident at Fukushima, it is necessary to ensure the safety of radiography for various professionals working together at a disaster site. We attempted to calculate the maximum number of cases that could be processed in one day in terms of individual dose limits in the 90° direction. In the Great East Japan Earthquake, it was difficult to examine more than 30 cases per day, even if examiners only performed dental radiography. Using our approach, examiners could process up to 119 cases, even at 0.5 m, if they were registered as a radiation worker. Based on public exposure, they could process up to 26 cases at 1.0 m, and 80 cases at 1.5 m. Therefore, all individuals, other than radiation workers, should stay 1.5 m or more away from the X-ray generator, if they do not use any protection materials, such as screens and aprons. The guidelines of Japan's Ministry of Health and Welfare on the safe use of X-ray equipment for home-care service in 1998 stated that healthcare workers other than an operator and a support person, family members, and caregivers should stay 2 m or more away. Moreover, a similar guideline for first-aid stations at disaster sites in 2009 stated that healthcare workers other than an operator and a support person, should stay 2 m or more away for occupational protection, or 3 m or more away for public protection. If radiological examiners adhere to these guidelines, they can take extra-oral plain dental radiographs safely even at disaster sites.

Compared to other methods of dental radiography, the use of plain dental radiographs in this study had some advantages. As compared to the intra-oral method, we do not need to open the mouth; we could record the dental information from outside if the dead body had mouth opening difficulty because of burnt skin, dried skin, rigor mortis and fragile teeth and jaws. Even if teeth and jaws were fragile, we could place the dead body in the prone position by using assistive devices. Additionally, three plain dental radiographs provided broader coverage than 10 intra-oral radiographs; the plain dental radiography would be less time-consuming than intra-oral radiography. Moreover, fewer radiographs and the prone position used in this method lead to a lower dose of scattered X-rays per case than the dose used in the intra-oral method, as previously mentioned. As compared to panoramic radiography, plain dental radiography could be used by adjusting the FPD position and the cone angle of the X-ray generator against the head, even if a head is bloated due to post-mortem changes, or is severely tilted upward by burnt muscles. In contrast, it is sometimes difficult to place maxillary and mandibular dental arches of a large sized head or a tilted head on the focal trough for panoramic radiography.[19] As compared to MDCT, plain dental radiography is not affected by the metal artefacts that often make it difficult to observe the teeth.

Moreover, plain dental radiography has some limitations. The resolving power of the plain dental radiographs was 3.0 lp/mm, which was less than that of intra-oral radiographs; if the characteristic points were very small, a detailed comparison with ante-mortem radiographs would be difficult. In addition, lateral oblique radiography and contact radiography are classical methods, which have been used since before CT was developed; ante-mortem radiographs are commonly taken by different approaches. When comparing the radiographs taken using such different modalities, we were not able to superimpose the images, because of different distortions and magnifications. It is necessary to compare the characteristic points in both post-mortem and ante-mortem radiographs. Yet, the FPD remains an expensive device. If they became popular in first-aid centres and general hospitals, such devices would be more easily obtained in future.

CONCLUSION

Extra-oral plain dental radiography can be used for disaster victim identification by dental methods, even when it is difficult to open the mouths of dead bodies. Portable and rechargeable devices, i.e. an FPD and a hand-held X-ray generator, are convenient to bring to and use at any mortuaries, even at disaster scenes lacking electricity and water. In future, we will study whether plain dental radiographs are sufficient to compare with ante-mortem radiographs in cases in actual practice.

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Semi-automatic forensic approach using mandibular midline lingual structures as fingerprint: a pilot study

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KEYWORDS

forensic identification, finger print, mandibular lingual canals, forensic odontology, victim identification

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ABSTRACT

Background: Previous research proposed the use of the mandibular midline neurovascular canal structures as a forensic finger print. In their observer study, an average correct identification of 95% was reached which triggered this study.
Aim: To present a semi-automatic computer recognition approach to replace the observers and to validate the accuracy of this newly proposed method.
Materials and methods: Imaging data from Computer Tomography (CT) and Cone Beam Computer Tomography (CBCT) of mandibles scanned at two different moments were collected to simulate an AM and PM situation where the first scan presented AM and the second scan was used to simulate PM. Ten cases with 20 scans were used to build a classifier which relies on voxel based matching and results with classification into one of two groups: “Unmatched” and “Matched”. This protocol was then tested using five other scans out of the database. Unpaired t-testing was applied and accuracy of the computerized approach was determined.
Results: A significant difference was found between the “Unmatched” and “Matched” classes with means of 0.41 and 0.86 respectively. Furthermore, the testing phase showed an accuracy of 100%.
Conclusion: The validation of this method pushes this protocol further to a fully automatic identification procedure for victim identification based on the mandibular midline canals structures only in cases with available AM and PM CBCT/CT data.

INTRODUCTION

Teeth have been widely used for forensic victim identification. Since the mandible is the strongest bone of the skeleton of the face, it is often preserved after death[1] which raises the interest in the potential value of mandibular bony fragments as identification methodology. The mandible contains many accessory foramina and canals, mainly on the lingual side, with anatomical variation among individuals.[2,3] The mandibular midline foramina are different in number, morphology, size and intraosseous canal structures.[4,5] These significant distinctions make these midline structures unique for a particular individual.[6]

Recent developments in dentomaxillofacial radiology such as Computer Tomography (CT) and Cone Beam Computer Tomography (CBCT) with high quality images and low dose aided in the extensive use of these technologies in the fields of dentistry and maxillofacial surgery. Moreover, some countries participating in war let the officers take a CBCT prior to departure to be used as an identification tool in case of death.[7] Combining these factors, previous research[7] was triggered in which the mandibular midline neurovascular canal structures was investigated as a forensic fingerprint. In their research, observers evaluated CBCT data of ante-mortem (AM) and post-mortem (PM) showing an average score of 95% correct identification.

The aim of this study was to present a semi-automatic computer recognition protocol to identify PM cases by comparing their mandibular midline canal structures to AM cases and to validate the accuracy of this approach.

MATERIALS AND METHODS

Ethical approval

Ethical approval was obtained from the Ethical Review Board of the University Hospitals Leuven (S57587). No informed consent was required for this retrospective study as no patients' identifiable data was disclosed.

Patient selection

CBCT/CT scans of 13 human mandibles were selected retrospectively from patients referred to the Dentomaxillofacial Radiology Centre (University Hospitals Leuven, Leuven, Belgium) with 10 cases having 2 scans with adequate image quality (no patient movement, no poor quality, etc.), no fracture in the lower jaw and no pathological lesions in the interforaminal region of the mandible. The mean difference between the 2 scans was 1.5 years. These 20 scans served as training data. Two of the 10 cases had a third scan at least one year later to the second scan and the last 3 cases had only 1 scan. These 5 scans were used as testing subjects to the protocol. The imaging modalities for these 25 scans were 4 CBCTs and one CT: 1. Accuitomo 170 (Morita, Kyoto, Japan), 2. Scanora 3D (Soredex, Tuusula, Finland), 3. ProMax (Planmeca OY, Helsinki, Finland), 4. NewTom VGi evo (QR s.r.l, Italy) and 5. Spiral CT scanner (Siemens Somatom Definition Flash; Siemens AG, Erlangen, Germany)

Matching protocol of AM and PM data

The general procedure to identify a PM to its corresponding AM is based on the assumption of having a database of AM mandibles then apply the protocol of the PM in question to all AM in the database until a correct classification is reached. The 2 available classes are: "Matched" and "Unmatched". The "Matched" class is when a correct identification is reached, while the "Unmatched" class is when no correct identification is reached. The general procedure of the matching protocol is explained as follows:

1. Region of interest (ROI) identification: which is the extraction of the mandibular midline canal region in three dimension (3D) as a volume.
2. Voxel based registration: apply a rigid voxel based registration procedure between the AM and PM to calculate a metric value.
3. Classification: measure the difference between the obtained metric value and the mean of each class (Matched vs Unmatched). The closer (lower) the difference value is to a class then it means that the AM belongs to that class.

Building the classifier

The 10 cases with 2 scans were used to build the classifier. The first scan of each case was considered the AM situation while the second scan was used to simulate the PM. Each PM was compared to each AM resulting into 100 comparisons. The target was to be able to group the correct matched scans (n=10) into a group totally separated from the rest of the cases that should not match (n=90).

The DICOM images of each scan were then imported into the Amira software (FEI, USA) where all the steps were implemented as follows:

1. Each ROI was identified by selecting the Pogonion point (Pog), i.e. the most anterior point on the mandibular. A window of width 9 mm with the Pog as the middle point and height starting from of the Menton (Me) which is the lowest point on the lower border of the mandibular symphysis, up to the Infradentale point (Id) which is the most superior anterior point on the mandibular alveolar process between the central incisors. Figure 1 shows an example of ROI identification process.

2. Each ROI was then resampled to have isotropic voxel size equivalent to the highest resolution. For example, if the voxel size of the case was 0.2x0.2x1mm, it is then resampled to 0.2x0.2x0.2mm.
3. Each PM was registered to each AM via rigid voxel based registration with mutual information and a corresponding metric value was then recorded.[8–10] The idea behind the volumetric matching algorithm is trying to align 2 objects based on information theory and similarities between these 2 objects. This metric has no unit of measurement and is closer to zero when the objects are not similar while higher values are found (closer to 1) when these objects are more similar to each other.
4. Each correct AM and corresponding PM metric values were grouped under "Matched" class (n=10) while all other pairs were grouped under "Unmatched" class (n=90).
5. The mean value of each class was calculated and used for the classification step to identify the PM belongs to which class. Figure 2 shows an example of a correctly "Matched" case and another correctly "Unmatched" case.

Testing and validation

After the classifier was built, the testing phase started. Two cases from the 10 subjects had a later scan of CBCT at least one year after the second scan. These were used to identify matched cases while the other 3 cases with single scan were used to identify unmatched cases. The matching protocol was applied to these 5 scans that served as testing subjects.

Statistical analysis

Data was analysed using the statistical software package MedCalc (Version 16.4.2, MedCalc Software, Ostend, Belgium). In a first step, the

Figure 1: Example of ROI identification.

(A) The whole mandible with the ROI surrounding.

(B) Frontal view of the ROI after extraction.

(C) Lateral view of the ROI after extraction.

(D) Midsagittal cut slice of the ROI showing the midline lingual structures.

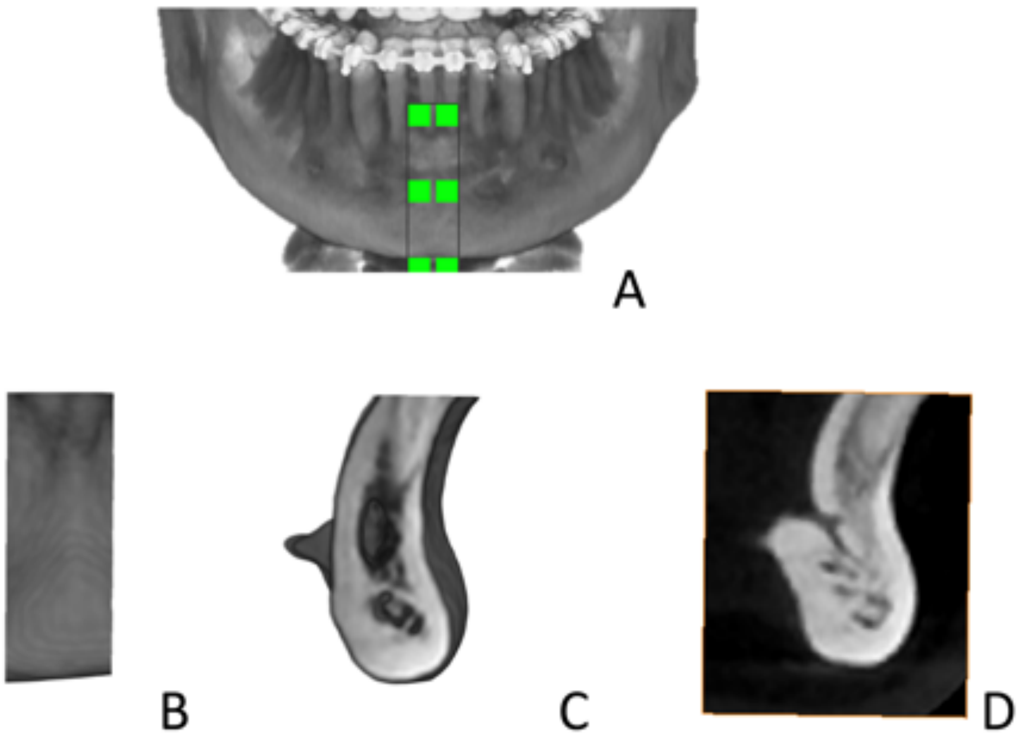


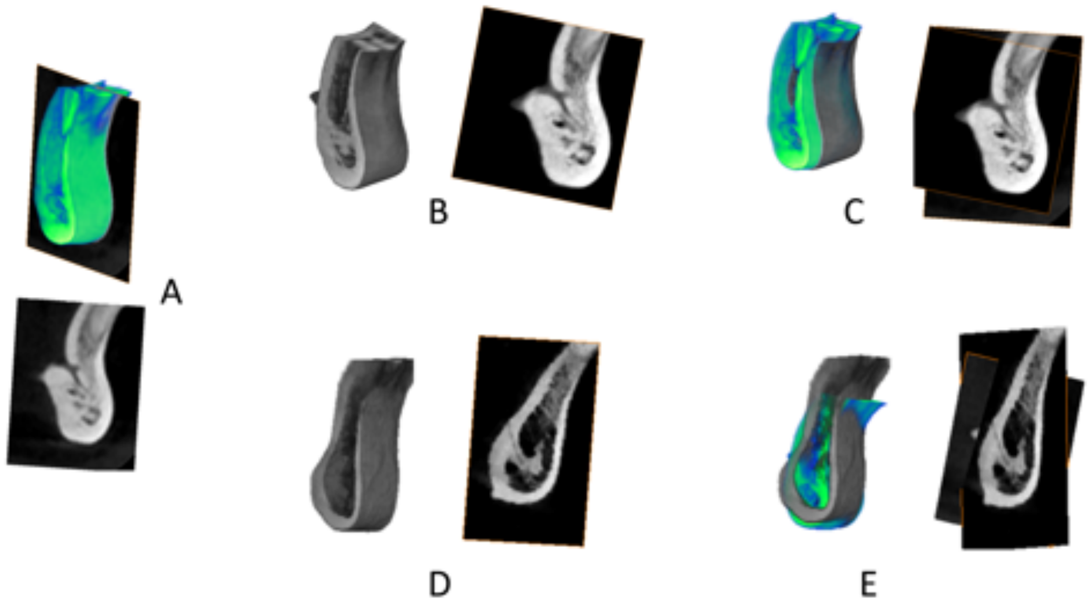
Figure 2: Example of classification (step 3 of matching protocol) with every ROI the midsagittal cut slice is shown.

(A) ROI of AM case shown in Figure 1.

(B) ROI of corresponding PM case.

(C) PM (B) to AM (A) registration showing perfect matching (metric value = 1).

(D) ROI of another PM that does not match. (E) PM (D) to AM (A) registration showing no matching (metric value = 0.4)



Shapiro-Wilk test was performed to validate the normality assumption. For the "Matched" and "Unmatched" groups, the means, standard deviations, the 95% confidence interval of the mean and standard error of the means were calculated as the means were used for classification purposes. The unpaired t-test was applied to compare between the two classes. Statistical significance was set at a P-value ≤ 0.05. For the testing procedure, the accuracy, specificity and sensitivity were calculated.

Classification results

The Shapiro-Walk testing proved normality distribution of the measurements. Table 1 shows the descriptive results of the "Unmatched" and "Matched" groups and Figure 3 shows the bar plot of these groups. Statistical significant difference ($P<0.0001$) was found between the two classes with mean of metric values of 0.41 and 0.86 corresponding to "Unmatched" and "Matched" respectively.

Table 1: Descriptive results of the classes: "Unmatched" and "Matched"

	Sample size (n)	Mean	Standard Deviation	95% CI for the mean	Standard error of the mean	Unmatched vs Matched
Unmatched	90	0,41	0,11	0,3903 to 0,4373	0,01	P < 0,0001
Matched	10	0,86	0,15	0,7540 to 0,9700	0,05	

Fig.3: Bar plot of the two classes: “Unmatched” and “Matched”

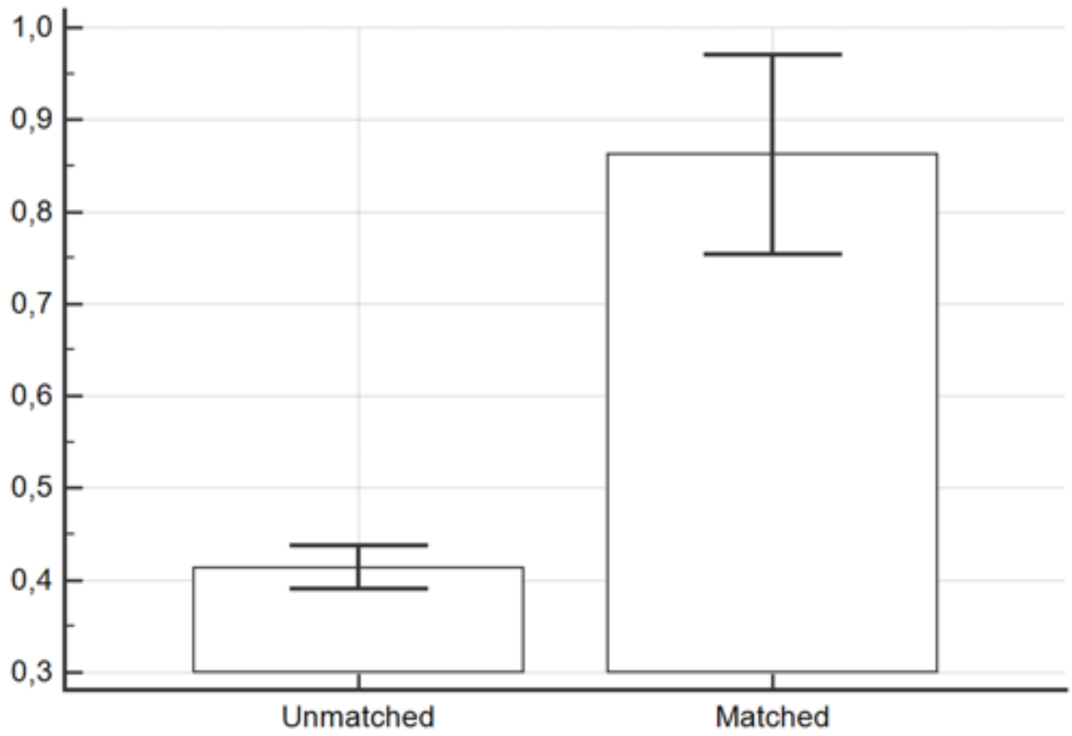


Table 2: Classification results of the 5 testing cases versus the 10 AM cases in the database.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
PTest1	1	0	0	0	0	0	0	0	0	0
PTest2	0	1	0	0	0	0	0	0	0	0
PTest3	0	0	0	0	0	0	0	0	0	0
PTest4	0	0	0	0	0	0	0	0	0	0
PTest5	0	0	0	0	0	0	0	0	0	0

Testing results

Table 2 presents the classification results of the 5 testing cases when compared to the 10 AM cases in the database. It has to be noted that PTest1 was the third scan of P1 and the PTest2 was the third scan of P2. Therefore, based on this classification, the accuracy was found to be 100%, sensitivity and specificity were also 100%.

DISCUSSION

In this paper we presented a semi-automatic protocol to identify PM cases by comparing their mandibular midline canal structures to AM cases. The first aim was to build a classifier which was done based on 10 subjects. The results showed that the classes “Unmatched” and “Matched” were significantly different. The means, standard

deviations and 95% confidence intervals of the mean revealed no overlap between the two classes (Table 1, Figure 3). However, as with any classifier, outliers could be found where 3 out of the 100 matches were misclassified indicating an accuracy of 97%.
The second aim was to validate and test the accuracy of the classifier which was done using 5 scans. The testing phase revealed an accuracy of 100%. An example of a matching procedure was shown in Figure 2 with correct matching into the "Matched" class (Figure 2C) with metric value of 1 while the correct classification in Figure 2E into the "Unmatched" class was with metric value of 0.4.
Based on these results, the proposition that the mandibular midline canal structures are indeed unique and can be used in identification of victims when AM and PM CBCT/CT data are made available. Different factors would affect the accuracy of this proposed protocol such as imaging, ROI selection and sample size. As mentioned previously, 4 different CBCT systems and one CT were used for the building and testing phases. The use of different systems didn't affect the results and this could be explained by the use of voxel based registration with mutual information as the method relies on similarities between volumetric information. This method overcomes the errors that could rise if segmentation and surface/point based registration were used due to the difficulties of accurate segmentation from CBCT images[11] and the lower accuracy of surface or point based registration.[12] Another factor in imaging would be the voxel size, the smaller the voxel size, the better the resolution and thus more accurate results. In this study, most voxels were not isotropic and this would affect the registration, therefore, the resampling step was added to have isotropic voxel sizes. The largest voxel size in the z direction (slice thickness) was 1mm. Larger voxel sizes were not tested. Metal and filling artifacts[13] didn't play a role in this study as the region of interest is quiet far from such artefacts. Exceptional cases would be cases with mandibular trauma but they were excluded from this study or patients who underwent genioplasty, these also would be excluded.
The protocol was called semi-automatic because of the first step of ROI selection which was done manually. The cephalometric points identification was an attempt to improve this manual step towards automation which would require from the user to identify only 3 points (Pog, Me and Id). However, cephalometric point identification is prone to user error up to 2mm.[11,14,15] Nevertheless, the use of a bounding box to extract the ROI based on these points and with a window width of 9 mm was selected to overcome these possible errors. Then all the other steps can be easily automated with no user interference even the ROI selection step could be automated as shown by Codari et al.[11]
It is true that this study was a proof of concept built with only 10 matching cases and tested against 5 other scans, but the promising outcome of the automation method suggests the use of this procedure to build a larger database. The proposed method would be a useful tool to filter out cases to facilitate victim identification. Nevertheless, more tests are needed on larger datasets to further verify the accuracy of this procedure.

CONCLUSIONS

The mandibular midline canal structures may be used to facilitate victim identification when AM and PM CBCT/CT mandibular data are present. The outcome of the current research may serve as a basis for further studies on automated victim identification by means of this patient-specific anatomic structure as well as other potentially unique anatomical landmarks.

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An automated technique to stage lower third molar development on panoramic radiographs for age estimation: a pilot study

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ABSTRACT

Background: Automated methods to evaluate growth of hand and wrist bones on radiographs and magnetic resonance imaging have been developed. They can be applied to estimate age in children and subadults. Automated methods require the software to (1) recognise the region of interest in the image(s), (2) evaluate the degree of development and (3) correlate this to the age of the subject based on a reference population. For age estimation based on third molars an automated method for step (1) has been presented for 3D magnetic resonance imaging and is currently being optimised (Unterpirker et al. 2015).
Aim: To develop an automated method for step (2) based on lower third molars on panoramic radiographs.
Materials and methods: A modified Demirjian staging technique including ten developmental stages was developed. Twenty panoramic radiographs per stage per gender were retrospectively selected for FDI element 38. Two observers decided in consensus about the stages. When necessary, a third observer acted as a referee to establish the reference stage for the considered third molar. This set of radiographs was used as training data for machine learning algorithms for automated staging.
First, image contrast settings were optimised to evaluate the third molar of interest and a rectangular bounding box was placed around it in a standardised way using Adobe Photoshop CC 2017 software. This bounding box indicated the region of interest for the next step. Second, several machine learning algorithms available in MATLAB R2017a software were applied for automated stage recognition. Third, the classification performance was evaluated in a 5-fold cross-validation scenario, using different validation metrics (accuracy, Rank-N recognition rate, mean absolute difference, linear kappa coefficient).
Results: Transfer Learning as a type of Deep Learning Convolutional Neural Network approach outperformed all other tested approaches. Mean accuracy equalled 0.51, mean absolute difference was 0.6 stages and mean linearly weighted kappa was 0.82.
Conclusion: The overall performance of the presented automated pilot technique to stage lower third molar development on panoramic radiographs was similar to staging by human observers. It will be further optimised in future research, since it represents a necessary step to achieve a fully automated dental age estimation method, which to date is not available.

INTRODUCTION

Evaluating medical images for forensic age estimation is generally performed by expert human observers, such as radiologists or dentists. Their age estimations are mainly based on the developmental status of different anatomical structures depicted in the medical images. When teeth are still developing, the established methods mainly use panoramic radiographs for dental age estimation. In adolescents and subadults third molars are evaluated for age estimation, since the other elements of the permanent dentition are fully developed.[1] Numerous staging techniques have been developed to evaluate third molar growth and multiple methods have been reported that estimate age based on developmental stages allocated in reference populations.[2-5] Additionally, for age estimation in adolescents and subadults, it is recommended to evaluate a hand/wrist radiograph. In case the hand/wrist bones appear fully developed, the medial end of the clavicle should be assessed – either on radiographs or computed tomography.[6, 7] Although criteria have been described to discern one developmental stage from another, for teeth as well as for long bones or carpal bones, allocating a stage remains susceptible to inter- and intra-observer variability.[8-11]
Since variability in stage allocation is caused by the human eye and mind, automated staging techniques and related age estimation methods have been developed. Automated techniques require the software to (1) recognise the region of interest in the presented image(s)[12], (2) classify the presented degree of development into predefined stages and (3) estimate the age of the subject based on the allocated stage(s) and age related information from a reference population.[13] To develop software for automation or to teach existing software to recognise and classify specific shapes, a training data set is necessary.
Automatic third molar localisation software (step 1) has been developed for magnetic resonance imaging (MRI).[14] A regression random forest framework was used to predict the landmarks of the third molars in 3D MRI data. The aim of the current study was to find a way to develop automated algorithms for the second step, i.e. allocating an appropriate stage to the developing third molars. To simplify the problem in this pilot study, 2D data from panoramic radiographs were used and the focus was on the development of lower third molars.

MATERIALS AND METHODS

Study population and image analysis

Four hundred panoramic radiographs were collected from the patients' files at Leuven University Hospital, Belgium. Radiographs were selected based on the development of the lower left third molars (FDI 38). Two observers (J.D.T. and P.R.) allocated developmental stages (Table 1, Figure 1) to each lower left third molar in consensus. In case of disputes, a third observer (P.W.T.) acted as referee to decide on the allocated stage. Observer 1 (J.D.T.) had 5 years of experience evaluating skeletal and dental radiographs for age estimation. Observer 2 (P.R.) was recently introduced to the field of age estimation. Observer 3 (P.W.T.) had over ten years of experience in dental and skeletal age estimation. Images were displayed on a Samsung HD television (model UE32E45300W, resolution 1920 x 1080 pixels) or Phillips HD television (model 65BDL4050D, resolution 1920 x 1080 pixels). Images were imported and enhanced for optimal visualization of the considered third molar in Adobe Photoshop CC 2017 software and saved as psd-files.
A modified Demirjian's staging technique was used.[15] Two additional stages were added to the eight Demirjian stages. First, a crypt stage was included, as suggested by Gleiser and Hunt (1955) (stage I).[16] Second, an additional stage was defined based on apical closure (cfr. Gleiser and Hunt (1955) stage XV, Moorrees (1963) stage A ½ or Kullman (1992) root stage Aci).[17, 18] Moreover, Demirjian stage D was replaced with Gleiser and Hunt stage VII, crown completed. Finally, the order of criteria for Demirjian stage E was changed. Since some lower third molars are monoradicular, the presence of a calcified furcation was considered less important than relative root length.
Twenty radiographs per stage per sex were selected. Since only the radiographical appearance mattered, no restrictions were applied concerning age or biological origin of the subjects. Exclusion criteria for panoramic radiographs were insufficient image quality and severe bucco-lingual inclination of the considered third molar. The latter characteristic was most often encountered when third molars were suspected to be in stage 2 (Figure 2).

Figure 1: Representative examples of lower left third molars in each developmental stage. Third molars are depicted within their bounding box.

Figure 2: Excluded panoramic radiograph. The lower left third molar was suspected to be in stage 2, but it was expected that the software would not be able to recognise the stage due to its severe (out of plane) inclination

Table 1: Descriptive criteria for developmental stages of third molars (modification of stages defined by Demirjian (1973), Gleiser and Hunt (1955), Kullman (1992) and Moorrees (1963)).

Stage	Description
Stage 0	The crypt can be suspected in the jaw bone. Calcification of tooth tissue has not started.
Stage 1	A beginning of calcification is seen at the superior level of the crypt in the form of an inverted cone or cones. There is no fusion of these calcified points.
Stage 2	Fusion of the calcified points forms one or several cusps which unite to give a regularly outlined occlusal surface.
Stage 3	a) Enamel formation is complete at the occlusal surface. Its extension and convergence towards the cervical region is seen. b) The beginning of a dentinal deposit is seen. c) The outline of the pulp chamber has a curved shape at the occlusal border.
Stage 4	a) The crown formation is completed down to the cemento-enamel junction. b) The pulp chamber has a trapezoidal shape. The projection of the pulp horns gives an outline shaped like an umbrella top.
Stage 5	a) Beginning of root formation is seen in the form of a spicule. b) The root length is still less than the crown height. c) Initial formation of the radicular bifurcation is seen in the form of either a calcified point or a semi-lunar shape.
Stage 6	a) The calcified region of the bifurcation has developed further down from its semi-lunar stage to give the roots a more definite and distinct outline with funnel shaped endings. b) The root length is equal to or greater than the crown height.
Stage 7	The walls of the distal root canal are now parallel and its apical end is still partially open.
Stage 8	The walls of the root canal are now converging at the apex. The apical end is still partially open.
Stage 9	a) The apical end of the root canal is completely closed. b) The periodontal membrane has a uniform width around the root and the apex.

Image processing and training of the software

A region of interest around the lower left third molar was delineated by manually placing a rectangular bounding box on each radiograph using Adobe Photoshop CC 2017. This enabled the staging software to only capture the image information of the considered third molar, disregarding the surrounding information. In fact, this step mimicked step 1 (recognise the region of interest in the presented image) of a fully automated technique. Dimensions of the

bounding box were standardized to 240 pixels x 390 pixels, regardless of developmental stage. The long axis of the box was parallel to the tooth axis. The upper short side of the box was 2 mm cranially of the highest cusp tip. (Figure 3).
Subsequently, the Photoshop psd-files were imported into MATLAB R2017a software, which only processed the image information in the bounding box. The image contrast was enhanced using contrast-limited adaptive histogram equalisation[19] (MATLAB function adapthisteq with default parameter settings). Several linear and non-linear multi-stage classifiers (Linear and Quadratic Discriminant Analysis, Decision Trees, Support Vector Machines, Nearest Neighbour Classifiers, Ensemble Classifiers) were tested

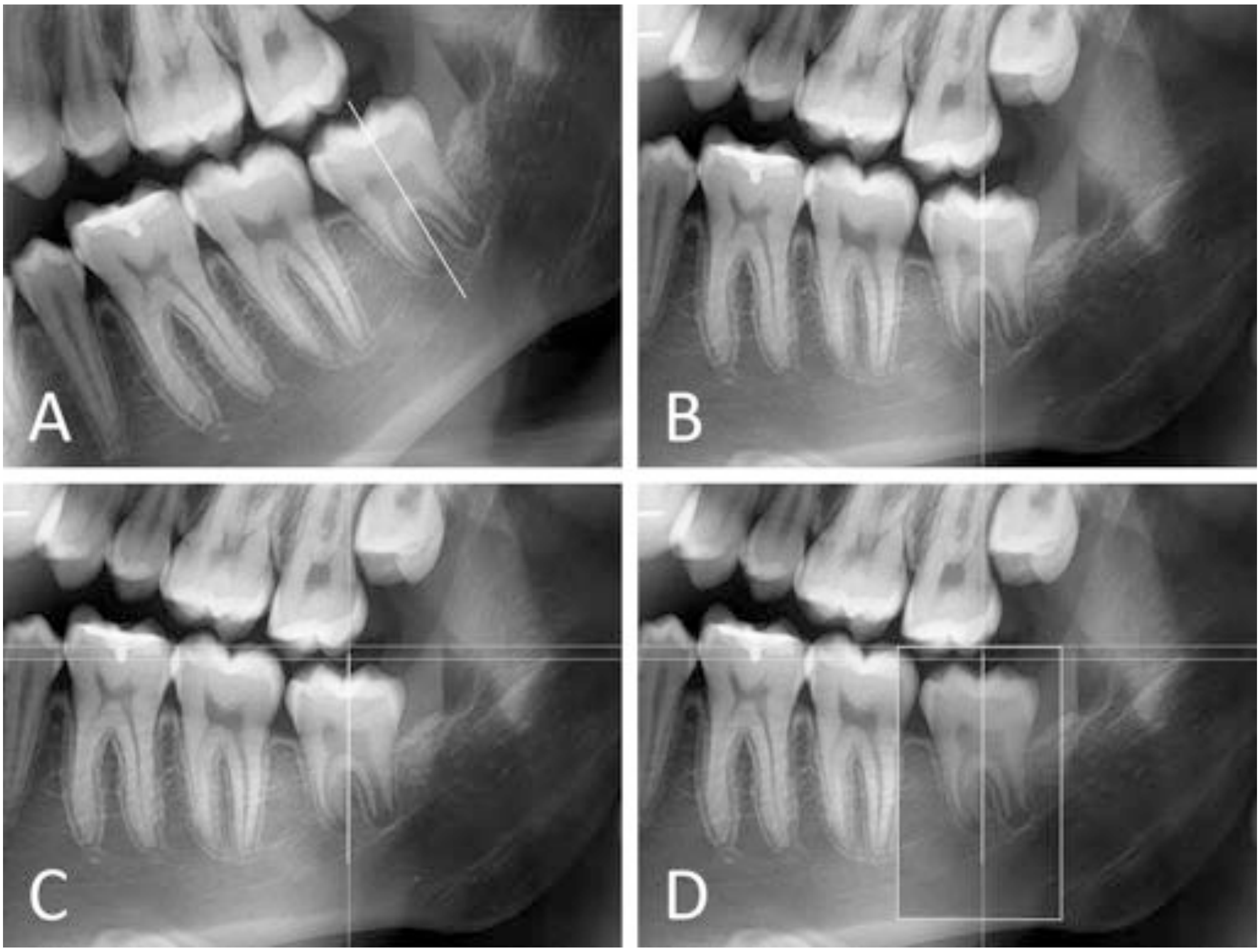
Figure 3: Close-up of image processing using Adobe Photoshop CC 2017.

(A) The long axis of the tooth was defined.

(B) The image was rotated to orient the long axis vertically.

(C) In succession, three guides were used to define the bounding box: (1) a vertical guide on the long axis of the third molar; (2) a horizontal guide touching the highest cusp tip; (3) a horizontal guide two millimetres occlusally from the previous guide.

(D) The top of the bounding box was on guide 3. The width was symmetrically divided around guide 1. For all third molars, dimensions of the bounding box were 240 pixels x 390 pixels, regardless of the developmental stage.



using the Classification Learner App of MATLAB R2017a after preliminary feature extraction and reduction from the region of interest (ROI) (first 10 Principal Components of appearance, Histogram of Oriented Gradients[20]) as well as a Bag of Features approach using SURF features[21]. Finally, a Deep Convolutional Neural Network approach was tested using transfer learning starting from AlexNet[22] (available as part of the MATLAB Neural Network Toolbox). AlexNet was originally trained on a subset of the ImageNet database[23], trained on more than a million images to classify images into 1000 object categories. As a result, the deeper layers of the network have learned a rich feature representation. The pre-trained AlexNet network was just adapted by replacing the final fully connected layer to train on the ten third molar stages. The weights were slightly retrained using stochastic gradient descent with momentum (momentum 0.9, initial learning rate 0.001, maximum number of training epochs 50 and a minibatchsize of 64).

Statistical analysis

For training and testing we used a 5-fold cross-validation, each round using 80% of the images for training and the remaining 20% for testing. Different metrics were calculated to measure the classification performance. The Rank-N recognition rate (Rank-N RR) measured how many times the correct stage (corresponding with the stage allocated by the human observers) was ranked first, second, ... N^{th} in the automated stage allocation process. Accuracy was defined by the first rank recognition rate, reflecting the percentage of correctly allocated stages. The mean absolute difference between the automated and manual staging (numbered 1 to 10) was calculated. In order to account for the ordinal character of the staging, linearly weighted Cohen's kappa and intra-class correlation coefficient (ICC) were calculated. Finally, confusion matrices obtained by cross-tabulation of the stages assigned by the software and the human observers (inter-observer agreement), allowed checking for systematic differences.

RESULTS

Using Transfer Learning as a type of Deep Learning Convolutional Neural Network approach outperformed all other tested approaches by at least 10% in classification accuracy. Table 2 shows the mean Rank-N RR, with a mean accuracy (Rank-1 RR) of 0.51. The mean absolute difference was 0.6 stages. The mean linearly weighted kappa was 0.82, ranging from 0.78 to 0.83 over the different cross-validation rounds. The mean ICC was 0.95, ranging from 0.93 to 0.96. From the cross tabulation (Table 3) it is clear that most frequently misclassified stages were in the neighbouring stages.

Table 2: Mean Rank-N recognition rate of automated stage allocation.

	The correct stage was ranked									
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth
Mean Rank-N RR	0.506	0.284	0.118	0.065	0.017	0.007	0.002	0	0	0

DISCUSSION

An automated technique to stage lower third molar development on panoramic radiographs was developed and its performance was tested. Inter-observer reliability (between automated and human observer staging) was similar to studies including only human observers. Although on average (only) 51% of the stages were correctly allocated, the mean absolute difference was 0.6 stages and the linearly weighted kappa coefficient 0.82, indicating that most misclassified stages were in the neighbouring stages only. Therefore, this novel approach looks promising and opens the perspective of countering variability in age estimation caused by intra- and inter-observer disagreement. After all, reproducibility was only in rare occasions reported to be perfect.

Table 3: Cross tabulation of allocated stages by the software (rows) and by the human observers (columns), normalised per row.

Stage	0	1	2	3	4	5	6	7	8	9
0	0.88	0.10	0.03	0	0	0	0	0	0	0
1	0.19	0.77	0.02	0.02	0	0	0	0	0	0
2	0	0.12	0.50	0.38	0	0	0	0	0	0
3	0	0.02	0.10	0.48	0.40	0	0	0	0	0
4	0	0.02	0	0.23	0.43	0.32	0	0	0	0
5	0	0	0	0	0.38	0.55	0.05	0.03	0	0
6	0	0	0	0	0	0.03	0.46	0.38	0.10	0.03
7	0	0	0	0	0	0.02	0.16	0.33	0.28	0.21
8	0	0	0	0	0	0	0.12	0.33	0.33	0.21
9	0	0	0	0	0	0	0	0.40	0.30	0.30

Observer- induced variability

In Table 4 statistics on intra- and inter-observer agreement were displayed for studies (2002-2017) that included assessments of developing third molars and that reported an appropriate statistic (more than merely the percentage of consistency). Notice that in these studies, staging as well as measuring was prone to disagreements. Whereas variability in measurements seems more random[24], variability in staging can be explained by multiple observer-related effects. First, systematic differences in allocated stages between research groups might be caused by a different calibration process. Kullman et al. (1996) reported that observers that were not calibrated showed significant variation in their registrations.[24] To our knowledge, no studies have been conducted to compare staging done by researchers from unrelated research groups.

Second, an observer might be uncertain about the stage to allocate. Corradi et al. (2013) attributed this to the tooth showing an intermediate stage of development or due to an unclear radiograph.[25] Instead of allocating the lowest stage – as originally proposed by Demirjian et al. (1973)[15] – they used artificial intelligence software to cope with what they called "soft evidence". The observer would then indicate two adjacent stages with the relative degree of belief per stage. By contrast, allocating one stage was defined as "hard evidence". Performance to discern minors from adults was better when soft evidence was incorporated than when only hard evidence was used.[25]

Finally, reproducibility is influenced by training. It has been stated that increasing experience, results in increasing reproducibility.[8, 24] To the limit, observer-induced variability could be circumvented by applying a deterministic automated staging technique. However, when supervised training of the neural network is applied – as was done in the current study – any errors in the supervised annotation of the training data will still be copied by the automated technique.

Automated age estimation methods

Automated methods imply exact reproducibility.[26] In the current study a staging technique was used, enabling a comparison between automated and human observer staging. However, when continuous data are considered (e.g. volumes of anatomical structures) and ROIs are determined automatically, this cannot be compared with any human observer action. In those studies, only the age estimation performance can be compared with that of methods based on human assessments. Some authors did study differences between volumes measured with different methods.

Table 4: Reproducibility statistics for the assessment of third molar development

Reference	Elements	Technique		Intra-observer agreement			Inter-observer agreement		
				Statistic		N	Statistic		N
Altalie (2014)[42]	Lower left permanent teeth and all third molars	S	Demirjian and Köhler respectively	Weighted kappa	0.99	100	Weighted kappa	0.91	100
Boonpitaksathit (2011)[43]	All third molars	S	Demirjian	Kappa	0.85-0.94	30	Kappa	0.69	30
Cameriere (2008)[4]	Lower left third molar	M	Cameriere	CCC	0.95-0.97	30	CCC	0.95	30
		S	Demirjian	Kappa	1.00	30	Kappa	0.93	30
Cameriere (2014)[44]	Lower right third molar	M	Cameriere	ICC	1.00	50	ICC	1.00	50
Corradi (2013)[25]	All third molars	S	Demirjian	Reproducibility index	0.79-0.89	1560	Reproducibility index	0.65-0.71	1560
Dhanjal (2006)[10]	Both left third molars	S	Demirjian	Kappa	0.70-0.78	73	Kappa	0.68	73
		S	Solari	Kappa	0.65-0.71	73	Kappa	0.56	73
		S	Moorrees	Kappa	0.61-0.65	73	Kappa	0.52	73
		S	Haavikko	Kappa	0.70-0.73	73	Kappa	0.64	73
Duangto (2016)[45]	Both lower third molars	S	Demirjian (modified)	Kappa	0.96-0.97	100	Kappa	0.92-0.93	100
Elshehawi (2016)[46]	All left permanent teeth and all third molars	S	Demirjian	Kappa	0.91	10	Kappa	0.77	10
Gunst (2003)[47]	All third molars	S	Köhler	Simple kappa coefficient	0.13	50	Simple kappa coefficient	0.14	50
Harris (2007)[48]	One lower third molar	S	Moorrees	Fleiss' kappa	0.96	225	-		
Hegde (2016)[49]	All third molars	S	Demirjian (modified)	Kappa	0.92	80	Kappa	0.94	80
Liversidge (2008)[50]	Lower left third molar	S	Moorrees	Kappa	0.77	100	-		
Liversidge (2010)[5]	Lower left third molar	S	Demirjian	Kappa	0.95	30	-		
		S	Moorrees	Kappa	0.91	30	-		
Lucas (2016)[51]	Lower left third molar	S	Demirjian	Kappa	0.88	15	-		

Maled (2016)[52]	All third molars	S	Demirjian	-			Kappa	0.85	167
Olze (2005)[53]	Lower left third molar	S	Demirjian	ICC	0.96	420	ICC	0.95-0.99	420
		S	Gustafson and Koch	ICC	0.89	420	ICC	0.87-0.98	420
		S	Gleiser and Hunt	ICC	0.98	420	ICC	0.95-0.97	420
		S	Kullman	ICC	0.94	420	ICC	0.92-0.98	420
		S	Harris and Nortje	ICC	0.90	420	ICC	0.83-0.93	420
Thevissen (2009)[54]	All third molars	S	Köhler	Weighted kappa	0.93	100	Weighted kappa	0.91-0.92	100
Thevissen (2011)[55]	Lower right third molar	M	Thevissen	WSCV	0.003-0.015	17	-		
Zandi (2015)[56]	All third molars	S	Demirjian	Kappa	0.91-0.93	250	Kappa	0.87-0.88	250
Zelic (2016)[57]	Lower left third molar	M	Cameriere	ICC	0.85	60	ICC	0.82	60

Dental age

Few studies on automated methods for dental age estimation have been conducted. None of them studied tooth development. Instead they were designed to estimate age in adults. Software for automated line-by-line scanning of tooth-cementum annulations (TCA) was presented by Czermak et al. (2006).[27] Segmentation was necessary and continuous data were used to estimate age. This method was tested on individuals of unknown age and compared with age estimation based on manual TCA counting, but no results on the comparison were reported. Since a comparison with a verified age was not possible, results were considered irrelevant for the current study.

Pulp/tooth volume ratios of monoradicular teeth were studied on cone beam computed tomography (CBCT) in patients between 10 and 65 years of age by Star et al. (2011).[28] Semi-automatic segmentation with Simplant Pro software allowed for the volumes to be defined. Differences between the real volumes and the volumes measured on the 3D images of the same teeth were calculated. For pulp and tooth respectively, they differed maximally 21% and 16%. Linear regression formulas were used with age as dependent variable and pulp/tooth ratio as predictor. The root mean squared errors from the regression model for incisors, canines, and premolars were respectively 12.86, 13.10, and 8.44 years.

Cameriere et al. (2015) studied canine pulp/tooth ratio in adults between 20 and 70 years old.[29] Using MATLAB, their method automatically segmented tooth and pulp tissue on radiographs of extracted teeth. It should be kept in mind that segmentation of in-situ teeth – especially at the root apex – becomes far more difficult due to the decrease in contrast, compared to extracted teeth. Age was estimated using a previously published formula[30], so it was not derived from the study sample. Mean absolute error was 3.05 years.[29]

The pulp chamber volume of upper and lower first molars was studied on CBCT by Ge et al. (2015).[31] Semi-automatic segmentation and voxel counting was performed using ITK-SNAP 2.4 software. A significant difference ($p = 0.024$) between the pulp volumes obtained from Micro CT and CBCT images was reported, with an average difference of 2.3%. The volume was incorporated into logarithmic regression to estimate age. Mean absolute error of 8.12 years and RMSE of 5.60 years were reported.[31] In a subsequent study the pulp of thirteen tooth types was studied in the same way. The pulp chamber volume of the upper second molar was found to be the most correlated with age.[32]

Skeletal age

Numerous fully automated (i.e. all steps in the process are computerised) methods for skeletal age estimation have been studied. Chang et al. (2003) developed an automatic bone age application that assessed phalangeal development in hand radiographs of patients aged 0.5 to 18 years.[33] Pre-processing (including segmentation) and assessment steps were fully automated. The software was trained using back propagation of neural network. The mean absolute error was less than 1.5 years in 84% of females and 79% of males.

Hand/wrist radiographs can also be evaluated using BoneXpert software (BoneXpert, v1.0; Visiana, Holte, Denmark, www.BoneXpert.com).[26] The software incorporates all three steps (feature recognition, stage allocation, age estimation) of the evaluation and can estimate age in girls from 2 to 15 years old and in boys from 2.5 to 17 years old. It automatically rejects images with abnormal bone morphology or insufficient image quality. The age estimation software was developed based on the findings of five human observers using the Greulich and Pyle atlas (GP)[34] or the Tanner and Whitehouse method (TW2).[35] Using leave-one-out cross-validation, they reported a standard deviation (SD) of 0.42 years between the chronological and estimated age for the GP method.[26] For the TW2 method, a corresponding SD of 0.80 years was reported. On average 68% of stages were allocated correctly to all bones by the software, ranging from 46% (ulna) to 83% (distal third phalanx).

Giordano et al. (2016) presented an alternative automated method based on TW2 for hand/wrist radiographs.[36] It was meant to estimate age in children up to six years old. Compared with two radiologists' assessments, 87%-91% of stages were allocated correctly. Mean absolute error was 0.37-0.41 years (SD 0.29-0.33) between the automated age estimate and the estimated age based on the radiologists' assessment.

The presented automated technique to stage lower third molar development on panoramic radiographs still has some shortcomings. Although it avoided the need for manual or automatic segmentation, a bounding box was necessary to fix the ROI of the stage allocation

Software to evaluate MR images is still being optimised. Since MRI provides a 3D depiction of the developing anatomical structures, analysis by the software is more complicated than based on 2D radiographs. To simplify the analysis Saint-Martin et al. (2014) predefined a limited region of interest at the epiphyseal-metaphyseal junction of the distal tibia on each MR image.[37] Their automatic method evaluated grey level variations in epiphyseal-metaphyseal fusion of the distal tibia and estimated age based on principal component analysis. Age estimation performance was only tested by checking whether or not individuals were correctly classified as minors or adults.

Urschler et al. (2015) developed an automated age estimation method based on the whole volumetric data of hand/wrist MRI.[38] It considers physeal fusion as a continuous process, so it does not use distinctive stages. A regression random forest framework is run by the software to pass decision trees taking the whole developmental process into account based on the training data.[13] Current medical computer vision and machine learning allows to process this complex data and use it for age estimation. Mean absolute error between chronological age and estimated age was 0.85 years (SD 0.58 years).[38]

Two major differences should be highlighted between the automated methods for skeletal age and those for dental age. In contrast to the dental age methods, the skeletal age methods were designed to estimate age in children, adolescents and subadults. Secondly, the performance regarding age estimation is better for the described skeletal methods than for the dental methods. A first explanation relates to the previous difference. Age estimation is more accurate and precise in younger individuals than in adults. A second explanation relates to the large variability in dental anatomy. Age related skeletal changes appear more homogenously on medical imaging than age related dental changes.

Limitations and future prospects

software. This bounding box can be automatically determined by adapting the detection method by Unterpirker et al. (2015).[14] This requires retraining the Random Forest Regressors on our set of panoramic images of third molars. Alternatively, one can (re)train a Deep Convolutional Neural

Network optimised for classification into third molar versus other objects and combine this with a sliding window object detection approach.
Another function that should be included in the process is automatic recognition of unsuitable images. As BoneXpert does[26], the software should be able to recognize images of insufficient quality and images in which the third molar appears extremely tilted.
Currently the highest stage difference between automated and human staging was three stages, but this only occurred once. It should be kept in mind that the current software analyses the image in a totally different way than the human observer does. It only relies on (implicit) non-localized appearance features that maximally distinguish the stages given the training set. This could be improved by replacing some of the fully convolutional layers by locally connected layers, taking positional differences into account. Furthermore, the current automated classification method does not explicitly consider the stage criteria (Table 1) as ordinal variables. Further improvements could be obtained by substituting the last classification layer using a classifier designed for ordinal classification. A two-step hierarchical/cascaded approach, first distinguishing between pre-root and root stages, followed by a final ordinal stage classification could be considered as well. This should be studied in future research, since large stage differences between automated assessment and human observer assessment are unacceptable for age estimation practice. Moreover, as with any Deep Convolutional Neural Network, the accuracy could certainly be increased when more panoramic radiographs are included to train the network. These additional radiographs could either be genuinely new training images or could be created through artificial data augmentation where data are added as transformed (translation, rotation, scale, contrast, etc.) copies of the original training data,.
Furthermore, the third step in the automated process, i.e. age estimation, needs to be implemented. Since the chronological age of the study population is known, the issue can be approached as a regression issue instead of a stage classification issue. This will allow to evaluate age estimation performance of the automated method, instead of stage allocation performance.
Finally, automated dental and skeletal methods should be integrated to further improve age estimation performance.[7, 39-41]

CONCLUSION

The overall performance of the presented automated pilot technique to stage lower third molar development on panoramic radiographs was similar to staging by human observers. Therefore, this novel approach looks promising. Optimisation of the technique will be conducted extending the current study sample and modifying the used software in further research. It represents a necessary step to render a fully automated dental age estimation method, which to date is not available.

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Age estimation by facial analysis based on applications available for smartphones

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ABSTRACT

Background: Forensic Dentistry has an important role in the human identification cases and, among the analyses that can be performed, age estimation has an important value in establishing an anthropological profile. Modern technology invests for new mechanisms of age estimation: software apps, based on special algorithms, because there is not interference based on personal knowledge, cultural and personal experiences for facial recognition.
Materials and methods: This research evaluated the use of two different apps: "How Old Do I Look? – Age Camera" and "How Old Am I? – Age Camera, Do You Look Like in Selfie Face Pic?", for age estimation analysis in a sample of 100 people (50 females and 50 males). Univariate and multivariate statistical methods were used to evaluate data.
Results: A great reliability was seen when used for the male volunteers. However, for females, no equivalence was found between the real age and the estimated age.
Conclusion: These applications presented satisfactory results as an auxiliary method, in male images.

INTRODUCTION

Forensic Dentistry has great applicability in the forensic field, through the study of specific characteristics that can differentiate people. Through their knowledge and methods for sex determination, age and ancestry,[1,2] we can highlight the age estimation of an individual, which presents an important expert function.

Anthropological examinations of the skull are used to estimate age, getting safe results (92% certainty) when compared to other bones of the skeleton, however, it is necessary ample knowledge of head anatomy and Forensic Anthropology, being the Odontologist the most qualified professional to carry out these studies, being able to even use techniques that go beyond the estimation of age by anthropometrics methods, such as facial recognition, dental elements or overlapping of images with scanned *ante-mortem* photographs with skull images in proportional scale. Besides that, in Brazil, Forensic Odontologists can act in different fields in Forensic Sciences and perform age estimation by facial images for in criminal cases.[3-7]

New research has pointed to a new method for the age estimation of an individual - facial analysis, since it is the main part of the visual examination of an individual and age as a crucial factor.[8]

Although the human visual system devotes specialized neural resources to face perception, age estimation through facial aesthetics can be affected by individual, cultural and social experiences, however, through the interpretation of software applications there is no interference based on personal knowledge, since only special algorithms are needed.[9,10] Also, due to rapid advances in computer graphics, facial age estimation, based on computer apps has become a particularly prevalent theme, because of the recent growth and development of technology, therefore, an essential goal of researchers in the field is to create automated facial recognition systems, which can be equal or even surpass human performance.[10]

It's possible to find apps on smartphones and tablets, since they are commercially available, so the present study aims to verify the use of two different apps "How Old Do I Look? - Age Camera" (Lucky Studio Games, USA) and "How Old Am I? - Age Camera, Do You Look Like in Self Face Pic?" (Liu Wang, China) in age estimation analysis.

MATERIALS AND METHODS

The research was approved by the Ethics Committee (CAAE 53719216.6.0000.5419), according to the requirements of Resolution 466/2012 (Brazilian National Health Council, 2012). The sample consisted of 100 individuals, between 18 and 60 years old, divided equally between female and male. Individuals who had the following characteristics in the region of face: inflammation, trauma, malformation, deformity, surgical scars, and who were not turns 18 at the time of data collection were not selected.

For photographing, a white background panel was used to standardize all portraits, and the same camera was used (Nikon Coolpix L810, Tokyo, Japan). Six front images of each participant were taken. The photos were taken with the height of the individual's eyes at the same height of the camera, perpendicular to the photographic beam, with a distance of 1,5m.[11] The background was always well illuminated (natural light of the day), avoiding shade projected at the white background panel, turning off the flash. The participant was instructed to remove caps, glasses or other loose items of clothing, keep their posture straight and arms closed to the body. From these photographs, three images presented a natural facial expression, and the others three, a smiling facial expression, so that a comparison was made between the ages estimated by both apps.

The age based on the photograph was estimated and recorded using two different apps: "How Old Do I Look? - Age Camera" – App A (Lucky Studio Games, USA) (Version 1.6, 2015, DeBuguer, USA), using Samsung Note 2 (Model GT-N7100, Andoid 4.3) and "How Old Am I?" – App B (Version 1.5, 2015, lemon Inc., China), using iPhone (Version 5, iOS9, Apple, Cupertino, CA, USA).

In this research we used univariate and multivariate methods to evaluate data. We performed the data analysis through two different statistical approaches. The idea was to verify how data behaves according each one. When we use univariate methods to handle data, average values are took into account and maybe they can hide important information. In the case of multivariate data, we are able to verify the values individually. Each age estimated by the photos (replicated three times for each person) was evaluated as a variable; this procedure provides more information about the system, allowing to observe the variation in data collecting regarding to the studied apps. Multivariate Analysis was executed by the Pirouette® package.[12-15]

The goal was to check how samples behave according to each situation:

Set 1: to verify results according to sex; training group was the entire set of results for women and men, using all replicates for the two applications in the studied features. Each sex was evaluated to verify how they behave individually.

Set 2: classification intended to check the possibility of separating the samples according to each software for each sex. Regression was used to check the coefficients by sex in each software.

Set 3: classification was used to check the possibility of separating the samples in relation to the feature for each sex. Regression intended to check the coefficients by sex in each feature.

For classification, the unsupervised learning was performed by means of PCA- Principal Component Analysis, which is a technique used to reduce the system dimension when there are many variables. A linear combination of the original variables is performed to generate a new axes system: the principal components (also called factors or latent variables). The purpose is to evaluate the natural similarities and the way the samples behave in clusters. Another way to verify classification is by supervised learning. In this case, we used SIMCA - Soft Independent Modeling of Class Analogies method.[18] The goal is to evaluate if each sample is correctly foresee in a class previously assigned. SIMCA is a technique that uses previous information to the analysis and it is recommended when there is more than ten samples for each class.[17,18] Classes are modelled by PCA and they give the information about how likely a sample is foresee into each class.

Partial least square (PLS) regression is commonly used to verify how a system with many independent variables are related to a specific property or observation, which corresponds to the dependent variable.[19-21] In the case of this work, the goal was to verify how ages collected by the apps fit to the dependent variable (real ages). Some indicators are important to verify the quality of results. In the PLS regression, there are two principal steps: validation and calibration. The method used in the first one was leave one out (LOO) cross validation, which consists of removing one of the samples from the set before perform the regression for the remaining ones. This procedure is repeated for all samples and provide the value of Q^2, which is correlation coefficient model cross validation. For calibration, values of the coefficient of the determination, related as R^2, must be evaluated. R^2 is used to give information about the linear correlation between the dependent and independent variables. This correlation is better as the value of R^2 increases. R^2 must be higher than Q^2.[18] The Root Mean Square Error of validation (RMSEV) and validation (RMSEC) must be compared as well, and RMSEC must be lower than RMSEV.

RESULTS

Set 1

The results for PCA (Figure 1) and SIMCA (Table 1) show that samples were well classified according to sex. In regression results for set 1 (Table 2) we observe the amount of information according to the number of optimal principal components for each regression (second column) and the statistical correspondences were obeyed, value of 0.68 for R^2 is achieved (Line 1). However, individual sex repression showed a poor correlation for women (R^2 0.12) whereas a good one was found for men (R^2 0.77). The same tendency was found for univariate regression (Table 3), which was performed over average values. The best correlation was found for men (R^2 0.72 for men, R^2 0.12 for women and R^2 0.65 for general).

Figure 1: PCA 3D-scores for Set 1.
W is the abbreviation used for female samples, here coloured as black. M is the abbreviation for male samples, here they are represented by red colour. PC1, PC2 and PC3 are the latent variables, which corresponds to the linear combination of the original ones.

Table 1: SIMCA results for set 1: classification errors for each gender.

	Preview in women's class	Preview in men's class
Women	43	7
Men	5	45

Table 2: PLS results for set 1

	# PCs	% cumulated variance	RMSEV	Q^2	RMSEC	R^2
General	2	99.12	5.59	0.66	5.48	0.68
Women	1	97.89	4.88	0.11	4.83	0.12
Men	2	99.44	5.97	0.74	5.72	0.77

#PCs = Principal Components; % cumulated variance = amount of information according to the number of optimal principal components; RMSEV = Root Mean Square Error of Validation; Q^2 = internal correlation coefficient model cross validation; RMSEC = Root Mean Square Error of Calibration; R^2 = correlation coefficient for calibration.

Table 3: Univariate Regression results for set 1

	Equation	R^2
General	$y = 0.76x + 5.73$	0.65
Men	$y = 0.37x + 12.78$	0.12
Women	$y = 0.70x + 9.77$	0.72

x = raw matrix; y = dependent variable

Set 2

In this case, each software was evaluated according to the sex. Red samples are regarding App B where as black ones belong to App A. Figure 2 A and B show the PCA results for women and men, respectively.

In SIMCA analysis, we observed that there is no good classification, indicating the absence of pattern for each software (Table 4).

The PLS analysis shows a slightly better result for App B since the errors are smaller for both sex and we observe poor values of correlation for women (R^2 0.14 for App B and R^2 0.10 for App A) whereas better values are observed for men (R^2 0.78 for App B and R^2 0.75 for App A) (Table 5).

However, univariate regression made over medium values showed a better correlation (Table 6) for women (R^2 0.71 for App B and R^2 0.60 for App A) instead multivariate values. For men, all results were similar (Univariate Regression: R^2 0.71 for App B and R^2 0,72 for App A) (Tables 5 and 6).

Set 3

Data in set 3 were organized to evaluate both software methods according to different features: natural and smiling. Data were evaluated according to sex. PCA analysis (Figure 3) presents results for women in natural (red samples) and smiling (black samples) for both software (a) and the results for men (b).

In both cases of SIMCA classification (Table 7), no pattern was found and is not possible to separate samples according to each feature.
PLS and univariate regression are presented in Tables 8 and 9 respectively. The results showed that both applications failed to determine age for women in both natural and smiling (R^2 0.07 for natural and R^2 0.09 for smiling) (Table 8). However, for the men the result was satisfactory (R^2 0.76 for natural and R^2 0.62 for Smiling) (Table 8), being the natural one that presented less errors.

Figure 2: PCA results for set 2.

W is the abbreviation used for female samples and M is the abbreviation for male samples. App A is represented by the black colour and App B by the red colour

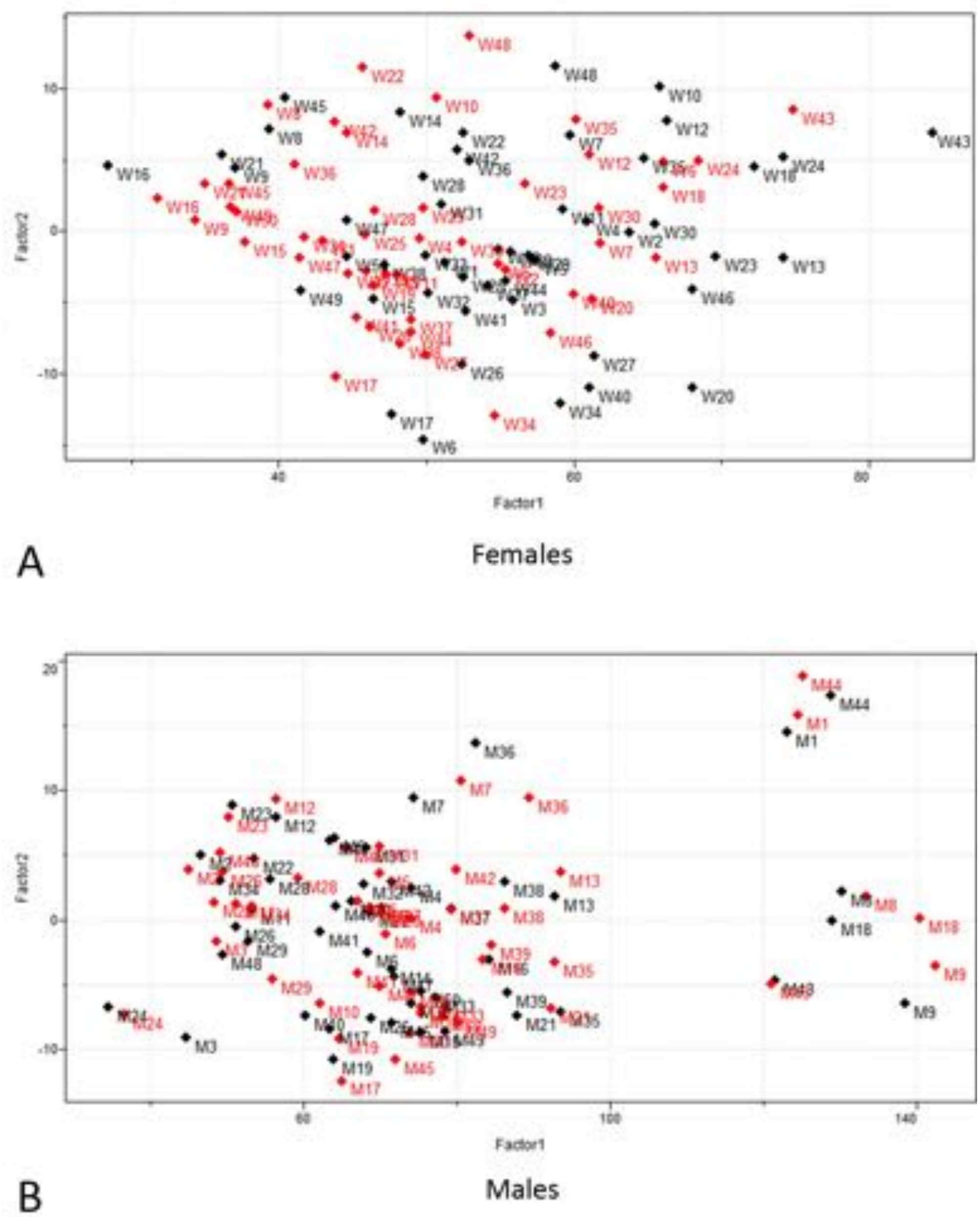


Table 4: SIMCA results for set 2

	App A	App B	Misclassified
Women			
App A	27	22	1
App B	22	28	0
Men			
App A	27	22	1
App B	17	32	1

App A = "How Old Do I Look? – Age Camera"; App B = "How Old Am I? – Age Camera, Do You Look Like in Selfie Face Pic?"

Table 5: PLS results for set 2

	# PCs	% cumulated variance	RMSEV	Q^2	RMSEC	R^2
Women						
App A	1	97.89	5.09	0.09	5.03	0.10
App B	1	98.21	4.88	0.13	4.83	0.14
Men						
App A	2	99.56	6.17	0.73	5.98	0.75
App B	2	99.49	5.89	0.75	5.61	0.78

#PCs = Principal Component; % cumulated variance = amount of information according to the number of optimal principal components; RMSEV = Root Mean Square Error of Validation; Q^2 = internal correlation coefficient model cross validation; RMSEC = Root Mean Square Error of Calibration; R^2 = correlation coefficient for calibration; App A = "How Old Do I Look? – Age Camera"; App B = "How Old Am I? – Age Camera, Do You Look Like in Selfie Face Pic?"

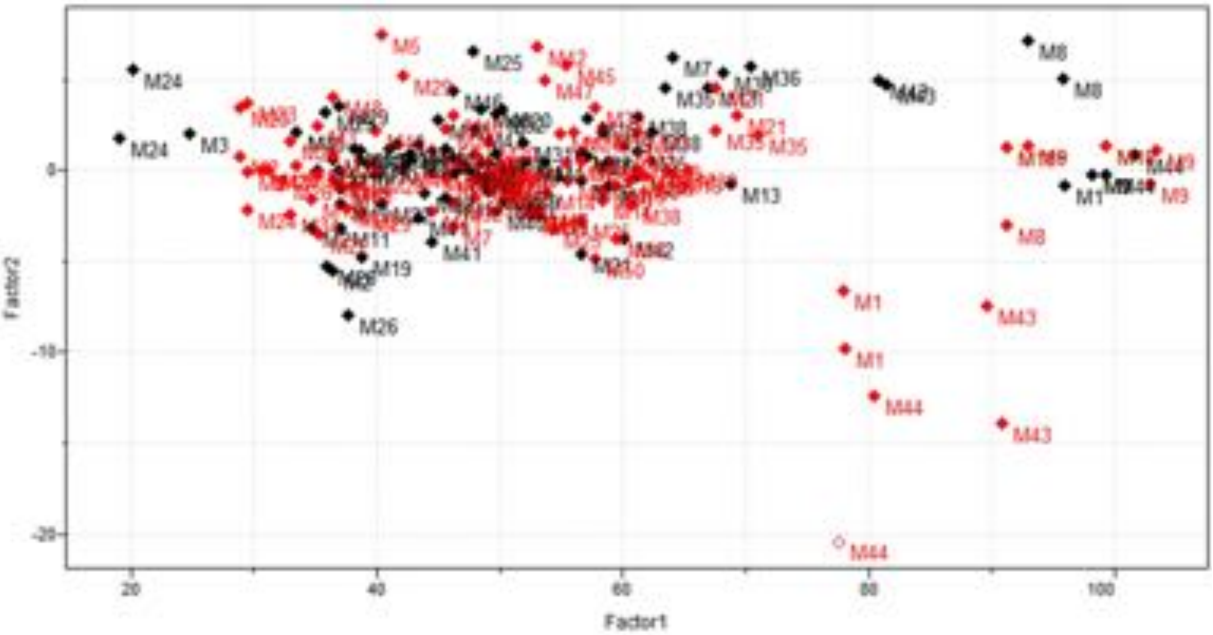
Table 6: Univariate Regression results for set 2

	Equation	R^2
App A	$y = 0.58x + 10.02$	0.60
App B	$y = 0.67x + 7.37$	0.71
App A	$y = 0.70x + 9.61$	0.72
App B	$y = 0.71x + 9.94$	0.71

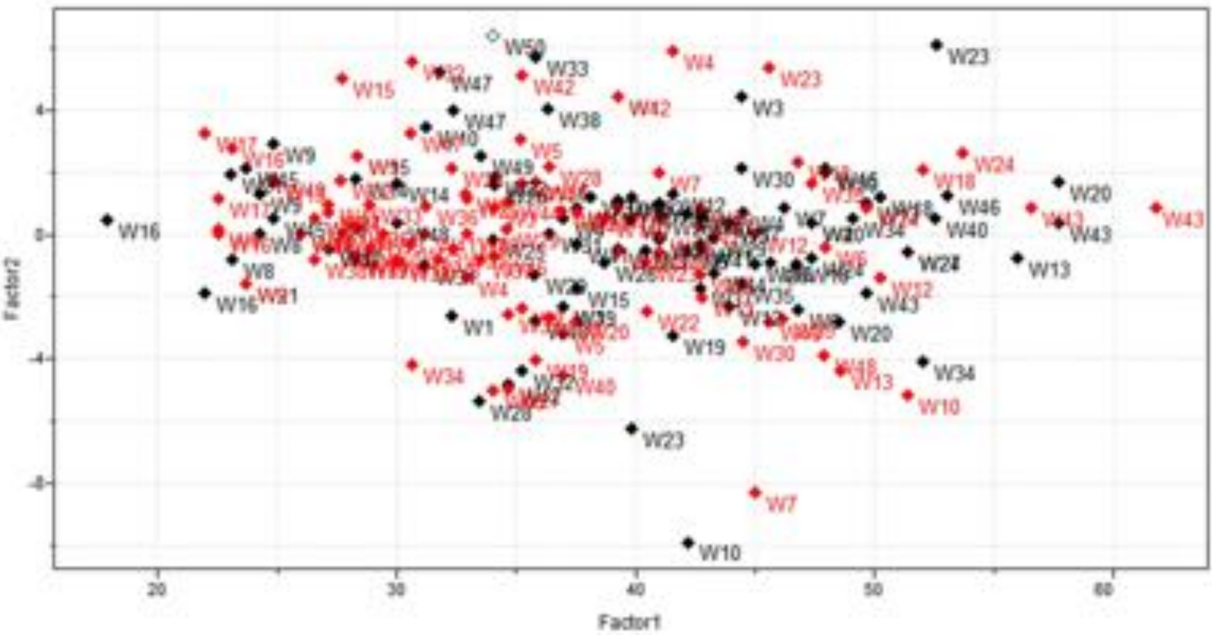
App A = "How Old Do I Look? – Age Camera"; App B = "How Old Am I? – Age Camera, Do You Look Like in Selfie Face Pic?"; x = raw matrix; y = dependent variable

Figure 3: PCA results for set 3.

W is the abbreviation used for female samples and M is the abbreviation for male samples. The smiling feature is represented by the black colour and the natural feature by the red colour



A Females



B Males

Table 7: SIMCA results for set 3

	Preview in Natural's class	Preview in Smiling's class	Misclassified
Women			
Natural	62	35	3
Smiling	56	40	4
Men			
Natural	62	37	1
Smiling	46	48	6

Table 8: PLS results for set 3

	# PCs	% cumulated variance	RMSEV	Q^2	RMSEC	R^2
Women						
Natural	1	99.31	6	0.07	5.65	0.07
Smiling	1	99.22	6	0.09	5.75	0.09
Men						
Natural	2	99.87	6	0.75	5.85	0.76
Smiling	2	99.82	8	0.60	7.44	0.62

#PCs = Principal Component; % cumulated variance = amount of information according to the number of optimal principal components; RMSEV = Root Mean Square Error of Validation; Q^2 = internal correlation coefficient model cross validation; RMSEC = Root Mean Square Error of Calibration; R^2 = correlation coefficient for calibration

Table 9: Univariate Regression results for set 3

	Equation	R^2
Natural	$y = 0.35x + 14.10$	0.07
Smiling	$y = 0.38x + 11.76$	0.09
Natural	$y = 0.77x + 7.61$	$R^2 = 0.76$
Smiling	$y = 0.64x + 11.94$	$R^2 = 0.58$

x = raw matrix; y = dependent variable

DISCUSSION

With the technological advances transforming life and routine, new forms of communication have been introduced through mobile devices, such as smartphones and tablets, and these devices have been presented as new means of socialization and communication through social networks and apps.[21] The task of estimating age aims to use computers algorithms to estimate the age of a person based on features extracted from the face image.[22]

When composing the samples for this study, we observed that both the apps put the facial images of the individuals in the same sex in which they were previously allocated, showing a standard in the division of classification of the samples according to the sex, independent of the facial expression or app, that is, there was a low number of sex classification errors, because others types of facial information, such as identity and sex, are more accurate when compared to the estimation of facial age, in which it's very challenging to accurately predict the age of a facial image, because the human facial aging is a slow process influenced by many internal and external factors, like the hair length, caps, glasses or other loose items of clothing, which can cover a lot of facial features related to the age estimates, besides that, the hair length can be a sex specific factor.[10]

When we tested the classification by app or facial expression, there was no standard in the separation of both, demonstrating that there isn't a feature that differentiates the results between the apps or facial expressions studied.

About the regression analysis techniques used in the study, to verify the correlation between the replicates and the real age of the individuals, when the results are separated only by sex, using all the replicates for the two apps and two facials expressions, we observed a correlation practically null between the age estimated and real age in women's data (R^2 0.12), and the highest correlations were observed for men's (R^2 0.77), which are responsible for influencing the general data and arriving at a reasonable correlation (R^2 0.68). This result can be justified by the fact that environmental factors, such as depressive symptoms, social class, social security and economic stability, have a greater influence on the visual estimation in women, when compared to the men's, influencing in the skin wrinkling and hair loss for example,[9] as Rexbye[23] observed in his study, that the effect of chronological age on perceived age in males is 3 years for visual age of 1 year, in females 2,5 for 1 year visually, a slightly greater effect. There is the hormonal factor too, that interferes with women's aging, because over time there is a decrease in oestrogen and progesterone levels, decreasing also the amount of collagen and elasticity of the skin, contributing to the wrinkling of the skin.[24] This fact can be explained because in women there are more factors that influence the apparent age, such as hormonal, genetic and environmental factors, which there are not for men.

When we observe the correlation of the estimated age by each of the facial expression in each sex, with both apps, there is still a low result for the women's, which does not occur in men, however, the natural expression presents a slightly higher of the correlation coefficient in both sex. This is not the case of the study by Sheretz[25], who photographed twenty-seven patients between 22 and 75 years old, males and females, with natural facial expression and smiling. This author found that there was no difference between the ages judged by the observers in both expressions, however, the ways of estimating the age of the individuals in the both studies cited were distinct, in addition to the sample with a superior mean age when compared with the mean age used in our study, which may also be a factor of divergence.[23,25,26]

Patzelt[11] verified the Photo Age app (Version1.5, © 2012, Percipo Inc., San Francisco, CA, USA) selecting 10 individuals (6 women and 4 men), of whom six photos were taken (three with the apparent smile and three with the natural face), these individuals were analysed by the cited app and by one hundred evaluator randomly selected to a future comparison. The subjects' real ages were between 42.1 ± 22.6 and the result found was: for the app between 43.1 ± 18.2; and for the evaluators between 41.5 ± 19.0. It has been demonstrated that the estimate age with the PhotoAge software app is a reliable procedure, with results comparable to the selected evaluators, besides the fact that it could be used as a method of estimating age. In our study, when we analysed the correlation between the ages estimated by each app in each sex (without the two facial expression) and the real ages of the individuals, we still found a low correlation for female, and a higher correlation for male, and we can even say that the App B presented a higher correlation coefficient. However, as we can see, the number of individuals in the different samples of the two studies cited are divergent, in the Patzelt[11] study ten individuals were selected six women and four men, and in the sample of our research, 100 individuals were selected (50 female and 50 male), being a relevant factor for the difference in correlation between the app studied and the real age of the individuals.

Therefore, considering the difficulties and limitations of the applications, it could be used as initial and auxiliary method in forensic investigations in the field of Forensic Dentistry, considering the importance of dentist´s knowledge in estimates of age by facial images for criminal cases, especially in male, because there is a significant correlation.

CONCLUSIONS

The results of this study show that the App B is slightly better for both sexes, compared to the App A but ultimately, both apps fail to determine the age for women, in natural and smiling photos. For men, the result was satisfactory, where the natural photo presented the least errors, thus it could be applied as an initial and auxiliary method for age estimation in images.

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Are cervical vertebrae suitable for age estimation?

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KEYWORDS

age estimation, cervical vertebrae, hand bones, third molars, method evaluation

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ABSTRACT

Background: The ability of cervical vertebrae (CV) staging to contribute in forensic age estimation is being discussed controversially. The large variability of CV geometries in the end stage of development might be the reason for not reaching a performance competitive to hand or third molar methods. Here we study the geometry of adult CV and demonstrate that the description of their "typical" appearance is often not met.

Materials and methods: Lateral cephalograms from clinical routine of 320 subjects aged 20 years or above (median 24 years, 52% female) were evaluated. The criteria for the end stage of CV development (Hassel-Farman, Baccetti) were examined by assessing them in terms of metric measurements: (1) rectangular shape of C3/C4, (2) at least one of the height-width ratios of C3/C4 >1 (both not <1), (3) significant concavities at the inferior margin of C2, C3 and C4. Metric data of the adults were also compared to those of 100 children aged 8-10 years (50% female).

Results: Adult CV often violated the criteria of rectangular shape (44% C3, 36% C4), of height-width ratio (16% C3, 35% C4) and inferior concavity (10% C2, 10% C3, 19% C4). All of the criteria for adult CV were fulfilled in only 24% of the subjects (95%CI 19-28%). The variability of measures of the CV shapes was large; e.g., the 95% reference ranges for the height-width ratios were 0.81-1.19 (C3) and 0.77-1.14 (C4). There was a material overlap of ranges of CV measures of adults and children.

Conclusion: While hand bones and teeth have well-defined appearances in the end stage of development, adult CV have a large biological variance of shapes; it is hard to define their "typical" appearance. Moreover, measures of CV geometry do not strictly separate adults from children. These facts might reason the limited usefulness of CV in age estimation.

INTRODUCTION

Dental age derived from the mineralization stages of teeth (for example Demirjian's classification[1]) and skeletal age assessed from the hand[2,3] are well established in forensic age estimation. In the recent time, the development of cervical vertebrae has been proposed to be used for the assessment of skeletal maturation and age estimation.[4-6] Part of the sources report clinical usefulness of cervical vertebrae.[7-9] Some authors are more careful and state it might be possible to use them,[10] whilst others are more brave and claim lateral cephalograms could replace hand radiograms.[11,12] However, the current discussion is controversial. There are a considerable number of reports of no or modest gain in using cervical vertebrae[13-15] and criticism of poor performance[16] and serious methodological flaws of such methods[17] and the suggestion to use other techniques for the assessment of skeletal development.[18] Thevissen and colleagues[19] as well as an Italian research group[20] proposed combined age estimation from teeth and cervical vertebrae instead of using cervical vertebrae alone.

The goal of this paper is to approach the question why age estimation from cervical vertebrae might be inferior to well established methods. This question is relevant for forensic odonto-stomatologists as well since age estimation should incorporate the combination several methods.[21] Optimal choice of the estimation method for dental age is hence not an isolated problem of odonto-stomatology. The point is rather which combination of dental and skeletal age estimation methods works best.

The development of cervical vertebrae is characterized by the change from a trapezoid towards a rectangular shape, by an increase of the height-width ratio, and the by the formation of the concavity at the inferior margin (see Figure 1). These characteristics are used by several staging systems.[4-6] However, this development is not as linear with a well-defined end stage as it is for the teeth[1] and the hand.[2,3] In our work with lateral cephalograms, we observed an apparently large variance in the geometry of adult cervical vertebrae. Therefore, we aimed at expressing the morphological description of cervical vertebrae having reached the end stage of development in terms of metric data, in order to replace subjective assessment with objective measurements. Here we present a quantitative analysis of the variability of the shapes of adult cervical vertebrae and a comparison with cervical vertebrae of children. Based on this examination, we propose an explanation why the use of cervical vertebrae might be not competitive to other methods in forensic age estimation.

Figure 1: Development of the cervical vertebrae in a sample individual. Lateral cephalograms were obtained at the ages of 9, 12, 15, 17 and 20 years. C3 and C4 change from trapezoids to a nearly rectangular shape, their height reaches and eventually exceeds their width, and the inferior margins of C2 through C4 which are initially flat develop a marked concavity.

MATERIALS AND METHODS

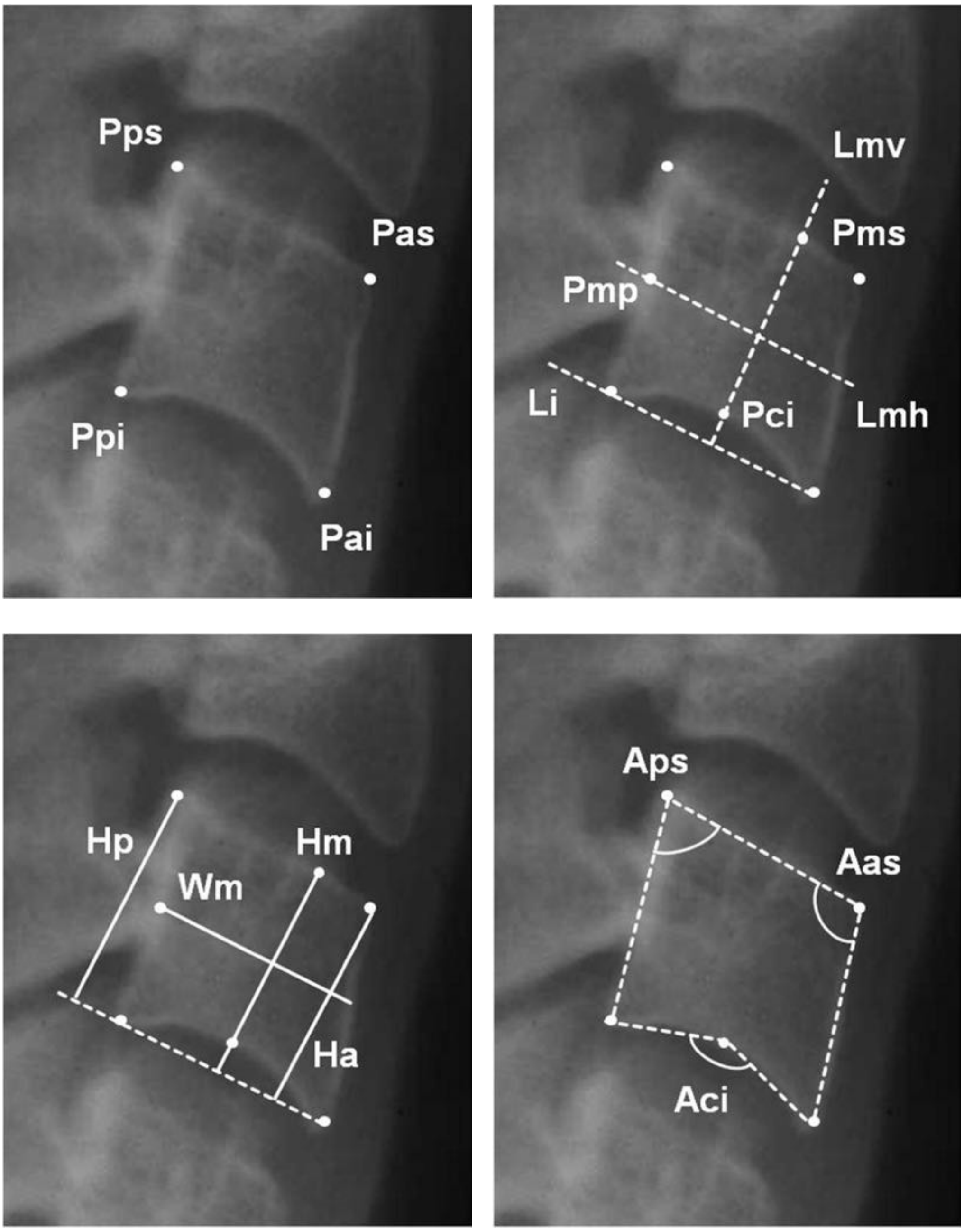
We analysed lateral cephalograms obtained in the clinical routine at the orthodontic department of the University Hospital Würzburg, Germany. In order to study the variability of adult cervical vertebrae shapes, we included a cross-sectional sample 320 adult subjects who were aged 20 years or older to ensure that the development of cervical vertebrae was finished and the end stage was reached. In order to examine the separation of adult cervical vertebrae shapes from those of children, we included a cross-sectional sample of 100 children aged from 8 to 10 years (before the pubertal growth spurt) for comparison. Patients

with syndromes that might affect skeletal development were not eligible. Of 442 selected radiographs, 22 were excluded due to image quality or superposition that might lead to difficulties in the precise evaluation.
The metric evaluation of the radiographs was carried out with the software OnyxCeph³ ™ (Image Instruments GmbH, Chemnitz, Germany). Landmarks on the images were set according to the definitions Table 1. Illustrations are provided in Figure 2. From the landmarks, the software calculated the following quantities that were then exported for statistical processing: inferior concavity angles of C2, C3, and C4; posterior, anterior and median height, median width, and posterior and anterior angle at the superior side of C3 and C4.

Table 1: Steps of the metric evaluation of cervical vertebrae (CV). The items with an asterisk (*) apply to all CV, the others only to C3 and C4

Symbol	Description	Construction
Pps	posterior superior vertex	free selection by observer
Pas	anterior superior vertex	free selection by observer
Ppi *	posterior inferior vertex	free selection by observer
Pai *	anterior inferior vertex	free selection by observer
Li *	inferior line	computed line through Ppi and Pai
Lmv	vertical median line	computed line perpendicular to Li through the midpoint of Ppi–Pai
Lmh	horizontal median line	computed line parallel to Li through the midpoint of Pas–Pai
Pci *	inferior concavity vertex	constraint selection by observer on Lmv and the inferior margin of the CV
Pms	superior median point	constraint selection by observer on Lmv and the superior margin of the CV
Pmp	posterior median point	constraint selection by observer on Lmh and the posterior margin of the CV
Ha	anterior height	perpendicular distance of Pas from Li
Hp	posterior height	perpendicular distance of Pps from Li
Hm	median height	perpendicular distance of Pms from Li
Wm	median width	distance of Pmp from midpoint of Pas–Pai
Ha/Hp	anterior-posterior ratio	computed from Ha and Hp
Hm/Wm	height-width ratio	computed from Hm and Wm
Aci *	inferior concavity angle	angle with arms Pci–Ppi and Pci–Pai
Aps	posterior superior angle	angle with arms Pps–Ppi and Pps–Pas
Aas	anterior superior angle	angle with arms Pas–Pps and Pas–Pai

Fig.2: Landmarks of the metric evaluation of cervical vertebrae



Inferior concavity angles of C2, C3, and C4, and median height-width ratio, anterior-posterior height ratio and superior side angles C3 and C4 were analysed. Criteria for the highest stage of development of cervical vertebrae as described, for example, by Hassel and Farman[4] or Baccetti and colleagues[5] were translated into terms of metric quantities as follows:

1. rectangular shape of C3 and C4 – considered fulfilled if the anterior-posterior height ratio was ≥0.9 (the anterior side of the trapezoid of children's C3/C4 is shorter than the posterior side), the posterior superior angle was ≥70 degrees and the anterior superior angle was ≤110 degrees (i.e. both angles differ from a right angle by no more than 20 degrees)

2. at least one of C3/C4 is rectangular in vertical shape (if not both, the second is squared) – considered fulfilled if the height-width ratio was ≥0.9 (this is quite liberal as ratios <1 indicate a horizontal rectangle)
3. significant concavities at the inferior margin of C2, C3 and C4 – considered fulfilled if the angles of the concavities were ≤160 degrees (note that reference images for established staging schemes suggest <150 degrees)

The statistical software SPSS 23 (IBM Corp., Armonk, NY, USA) was used for analysis. Summary data were presented by means and standard deviations. Comparison of male and female subjects was carried out by t-tests for adults and children separately. Frequencies and percentages of men, women and all subjects fulfilling the criteria for adult shapes of cervical vertebrae were computed, and rates of matching were displayed at the level of single measures, all measures of each of the cervical vertebrae, and perfect match (all measures of C2, C3 and C4). To characterize the variability of the geometry of cervical vertebrae and the overlap of adults and children, 95% ranges (2.5th to 97.5th percentile) of all measures were presented for male, female and all subjects. In addition, cumulative distribution functions were computed and displayed in diagrams.

For quality control, repeat measurements with a time-lag of two weeks were carried out on 50 lateral cephalograms to assess the intra-observer agreement (statistically expressed by the intra-class correlation coefficient, ICC).

The local ethics committee at the Medical Faculty of the University of Würzburg has confirmed that, according to the applicable legal and regulatory requirements in Germany, no ethical approval is needed for this research in the given setting (reference number 20170317-01).

RESULTS

Regarding data quality, reproducibility was excellent for height-width ratios, anterior-posterior height ratios, and inferior concavity angles (ICC from 0.95 to 0.99), good for the posterior superior angle of C3 and the superior angles of C4 (ICC from 0.88 to 0.92), and acceptable for the anterior superior angle of C3 (ICC 0.83).

Table 2 displays the averages of the metric data in our sample. In adults, the mean angles of the inferior concavities were ranging from 149 to 156 degrees which is close to the amount suggested by reference images in the literature. The mean height-width ratios were slightly (C3) or materially (C4) below 1, indicating that the "average" adult C3 and C4 is not a rectangle in vertical shape. Regarding the measures of rectangularity, the mean anterior-posterior height ratios of C3 and C4 were about one standard deviation below 1 in both men and women, and the mean deviations from a right angle of the angles at the superior vertices were ranging from 12 to 18 degrees.

Women had on average more pronounced inferior concavities than men. This was the only difference between sexes in adults with high significance ($P<0.001$ for C2, C3 and C4). With the exception of the anterior superior angle of C4, there were no other significant differences between adult men and women. In children, the means of the height-width ratios and part of the measures of rectangularity were closer to the adult values in girls than in boys, probably due to their earlier development. Of note, no significant differences between boys and girls were found for the inferior concavities of all three cervical vertebrae.

Table 3 lists the percentages of adults who fulfilled the metric criteria for adult cervical vertebrae. The inferior concavity angle is 160 degrees or below in 90% of the subjects for C2 and C3 and in 81% for C4. This means that 10% of the adult C2 and C3 and 19% of C4 might possibly not be considered to be typically adult with respect to their inferior concavity. The height-width ratio does still more often not match the description of adult cervical vertebrae. Every sixth C3 and every third C4 did not reach a ratio of 0.9 or above. When more strictly applying Baccetti's[5] description of the highest stage of development (at least one of C3/C4 is a vertical rectangle, the other is at least a square), we might require that both height-width ratios are ≥0.9 and at least one is >1. These criteria would be met by only 147 subjects (46%; 42% of men, 49% of women). The criteria for rectangular shape were fulfilled in less than two-thirds of the C3 and C4, which was most frequently attributable to a ratio of anterior and posterior height below 0.9.

Only one out of four subjects fulfilled all criteria (18% of men, 29% of women, $P=0.02$ for difference between sexes). The criteria for the inferior concavity were more frequently met by women ($P=0.02$ for C2, $P=0.01$ for C3, $P=0.03$ for C4), while men and women did not significantly differ with respect to the height-width and anterior-posterior height ratios (P-values from 0.29 to 0.82).

Table 2: Subject characteristics

ADULTS	**Men**	**Women**	**P-value**
Number of subjects	153	167	–
Age [y]	26.8 (7.8)	27.0 (7.4)	–
20 to <25 years – number (%)	87 (57)	92 (55)	–
≥25 years – number (%)	66 (43)	75 (45)	–
C2: inferior concavity angle [°]	153.2 (6.5)	149.5 (6.7)	<0.001
C3: height-width ratio	0.992 (0.100)	0.987 (0.093)	0.64
C3: anterior-posterior height ratio	0.933 (0.066)	0.945 (0.067)	0.12
C3: posterior superior angle [°]	76.4 (4.0)	75.6 (4.1)	0.11
C3: anterior superior angle [°]	107.2 (4.2)	107.8 (3.1)	0.17
C3: inferior concavity angle [°]	152.4 (7.0)	149.4 (6.6)	<0.001
C4: height-width ratio	0.934 (0.091)	0.943 (0.093)	0.38
C4: anterior-posterior height ratio	0.933 (0.070)	0.931 (0.063)	0.73
C4: posterior superior angle [°]	77.9 (4.8)	77.6 (4.1)	0.57
C4: anterior superior angle [°]	104.8 (4.4)	105.9 (3.8)	0.01
C4: inferior concavity angle [°]	156.0 (5.9)	151.8 (6.6)	<0.001
CHILDREN	**Boys**	**Girls**	**P-value**
Number of subjects	50	50	–
Age [y]	9.2 (0.5)	9.2 (0.6)	–
8 to <9 years – number (%)	19 (38)	14 (28)	–
9 to ≤10 years – number (%)	31 (62)	36 (72)	–
C2: inferior concavity angle [°]	173.5 (4.7)	172.6 (6.5)	0.43
C3: height-width ratio	0.614 (0.083)	0.679 (0.072)	<0.001
C3: anterior-posterior height ratio	0.672 (0.099)	0.756 (0.116)	<0.001
C3: posterior superior angle [°]	68.5 (5.7)	73.0 (4.4)	<0.001
C3: anterior superior angle [°]	117.2 (5.3)	113.1 (4.6)	<0.001
C3: inferior concavity angle [°]	173.5 (4.6)	173.8 (4.6)	0.72
C4: height-width ratio	0.627 (0.088)	0.666 (0.077)	0.02
C4: anterior-posterior height ratio	0.693 (0.126)	0.719 (0.100)	0.25
C4: posterior superior angle [°]	69.2 (5.4)	74.1 (5.7)	<0.001
C4: anterior superior angle [°]	113.6 (5.4)	112.0 (5.0)	0.13
C4: inferior concavity angle [°]	174.3 (4.5)	174.0 (4.1)	0.73

Data are means and standard deviations except for age groups which are N and %.

Table 3: Numbers of adults matching the criteria for adult CV geometry

Criteria		Total	Men	Women
C2: inferior concavity angle	≤160°	289 (90%)	132 (86%)	157 (94%)
C3: height-width ratio	≥0.9	268 (84%)	126 (82%)	142 (85%)
C3: rectangular shape		178 (56%)	84 (55%)	94 (56%)
anterior-posterior height ratio	≥0.9	227 (71%)	105 (69%)	122 (73%)
posterior superior angle	≥70°	297 (93%)	143 (93%)	154 (92%)
anterior superior angle	≤110°	234 (73%)	113 (74%)	121 (72%)
C3: inferior concavity angle	≤160°	287 (90%)	130 (85%)	157 (94%)
All criteria for C3 fulfilled		146 (46%)	63 (41%)	83 (50%)
C4: height-width ratio	≥0.9	209 (65%)	95 (62%)	114 (68%)
C4: rectangular shape		204 (64%)	102 (67%)	102 (61%)
anterior-posterior height ratio	≥0.9	220 (69%)	110 (72%)	110 (66%)
posterior superior angle	≥70°	308 (96%)	143 (93%)	165 (99%)
anterior superior angle	≤110°	283 (88%)	139 (91%)	144 (86%)
C4: inferior concavity angle	≤160°	259 (81%)	116 (76%)	143 (86%)
All criteria for C4 fulfilled		119 (37%)	53 (35%)	66 (40%)
All criteria for C2, C3, C4 fulfilled		76 (24%)	27 (18%)	49 (29%)

Table 4 shows the 95% ranges (i.e. the intervals from the 2.5th to the 97.5th percentile) of each measure in adults compared to children for males, females and all subjects. With the exception of the height-width ratio of C3 in males and the inferior concavity angle of C3 in females, the intervals for adults and children do overlap. This means that there are values of these measures that can occur in adults as well as in children and, since 95% ranges are presented, this fact is not attributable to single outliers.

More comprehensive information on the amount of overlap can be read from the cumulative distribution functions in Figure 3. For each value x on the horizontal axis, the corresponding percentage on the vertical axis says which part of the population has a value ≤x. The vertical lines mark the most extreme values for adults and children, and the corresponding percentages marked by the horizontal lines allow concluding about the extent of overlap. For example, in the first diagram referring to the inferior concavity angle of C2, the maximum value of adults was 172 degrees, and 35% of the children had a value below this limit. The minimum value of children was 153 degrees, 59% of the adults had a value below this limit, and hence, 41% had a value below. Thus, 41% of the adults and 35% of the children had their values within the intersection interval of the adults' and children's ranges of measurements.

DISCUSSION

Our analyses revealed a large variance in the appearance of adult cervical vertebrae and a high percentage of adult individuals whose shapes of cervical vertebrae disagree with descriptions of the end stage of their development.[4,5] To illustrate this, Figure 4 shows four examples of different patterns of not matching the criteria. The first case (female, 22 years) has very small height-width ratios of 0.67 (C3) and 0.78 (C4), and the shape of C3 cannot really be called rectangular (anterior superior angle 112 degrees).

Table 4: 95% ranges (2.5th to 97.5th percentile) of metric characteristics of cervical vertebrae in adults and children

Characteristic		Adults	Children
C2: inferior concavity angle [°]	male	139.2-165.0	162.5-180.0
	female	136.4-163.1	153.5-180.0
	all	137.0-163.7	155.2-180.0
C3: height-width ratio	male	0.804-1.210	0.440-0.788
	female	0.796-1.177	0.545-0.851
	all	0.806-1.193	0.475-0.812
C3: anterior-posterior height ratio	male	0.810-1.073	0.460-0.869
	female	0.818-1.074	0.547-1.010
	all	0.817-1.073	0.509-0.971
C3: posterior superior angle [°]	male	69.0-85.3	53.5-79.2
	female	66.1-82.2	63.8-86.1
	all	67.4-84.5	57.6-82.4
C3: anterior superior angle [°]	male	99.1-115.3	106.3-129.6
	female	101.5-113.7	105.2-125.1
	all	100.2-114.8	106.1-126.8
C3: inferior concavity angle [°]	male	139.7-167.8	159.8-179.8
	female	138.2-162.5	163.5-179.8
	all	138.7-167.3	163.4-179.8
C4: height-width ratio	male	0.772-1.138	0.472-0.875
	female	0.769-1.150	0.499-0.860
	all	0.772-1.137	0.484-0.872
C4: anterior-posterior height ratio	male	0.760-1.073	0.464-1.032
	female	0.820-1.049	0.480-0.913
	all	0.813-1.058	0.467-0.912
C4: posterior superior angle [°]	male	67.4-87.1	59.6-80.0
	female	70.2-86.2	61.2-87.4
	all	68.5-86.5	60.5-84.0

C4: anterior superior angle [°]	male	96.3-114.7	103.9-128.2
	female	98.9-114.3	101.0-122.0
	all	97.2-114.4	103.7-122.8
C4: inferior concavity angle [°]	male	144.0-167.6	161.9-179.7
	female	140.1-164.3	163.0-179.4
	all	140.6-166.3	163.1-179.4

In the second case (male, 25 years), the shapes of C3 and C4 are quite nicely rectangular (which is seen rather in a minority of the individuals), but the depths of the concavities are less than one-half of those shown in reference images (angles about 167 degrees), and the height-width ratios are 0.87 and 0.83, respectively. The third individual has a bit more pronounced inferior concavities, but again, the height-width ratios are not adult (0.79 and 0.73), and the anterior-posterior height ratios of 0.75 and 0.81 do not support a rectangular shape. In the last example, the inferior concavity angles (144 to 148 degrees) and the height-width ratios (1.15 and 1.19) represent adult values, but C3 is a typical trapezoid rather than a rectangle (anterior-posterior height ratio 0.83).

The large variability of the shapes of adult cervical vertebrae is probably a major reason for their lower performance when compared to other methods of age estimation. For example, in the end stage of development, the epiphyses of hand bones are joined with the metaphyses and the epiphyseal line is eventually no longer recognizable.[2,3] For teeth, the end stage is characterized by the closed apex.[1] In both cases, there is no variability in the appearance of these anatomic structures. In contrast, cervical vertebrae have many possible end points of their development and, therefore, it is difficult to determine from a radiograph which part of the way to the end point has already been passed.

In general, there are many anatomic measures are highly correlated with age but not all of them are suitable for age estimation. For example, body height or the waist-hip circumference ratios do materially change with increasing age, and their mean values are different between the age groups and sexes with high statistical significance. However, these measures have a high variability at the individual level which makes them unsuitable for forensic age diagnostics. The same argument might possibly apply to cervical vertebrae.

A second disadvantage seems to be the considerable overlap of the ranges of metric characteristics of cervical vertebrae of adults and pre-pubertal children. This situation is not found in hand bones where the epiphyses and metaphyses are not connected in children aged 10 years or below, and the epiphyseal lines are completely closed and disappear in most cases in adults aged 20 years or above.[2,3] For third molars, Demirjian's stage[1] is usually A to C (or only a crypt is present) in children aged 10 years or below, and G or H (rarely lower) in young adults. Now consider a real-world situation of forensic age estimation where the challenge is not distinguishing between adults and pre-pubertal children, but the diagnosis whether a young violator who is apparently about 16 years old and who claims to be 13 years old has passed the age of criminal responsibility (which is 14 years in Germany and many other countries). It is not hard to imagine that this type of diagnosis is difficult with a measure that does not strictly separate individuals aged 10 and 20 years.

The limitations discussed above do not imply that cervical vertebrae should be abandoned in general. Cameriere and colleagues[22] investigated age estimation from the ratio of the lengths of the anterior and the posterior side of C4. The mean absolute errors they reported indicate inferiority compared to age estimation from hand atlas methods.[2,3] However, the data presented in their paper suggest that the ratio under consideration increases rapidly around the age of 10 years. Hence, this measure might be valuable in the diagnosis of criminal responsibility in countries were the age threshold is 10 years. We should not forget in our debates the possibility that certain tools might be not recommended in general, but are particularly useful in distinct situations.

Fig.3: Cumulative distribution functions (CDF) of metric characteristics of cervical vertebrae in adults (black curve) and children (grey curve). Dashed lines mark the overlap of children's and adults' ranges

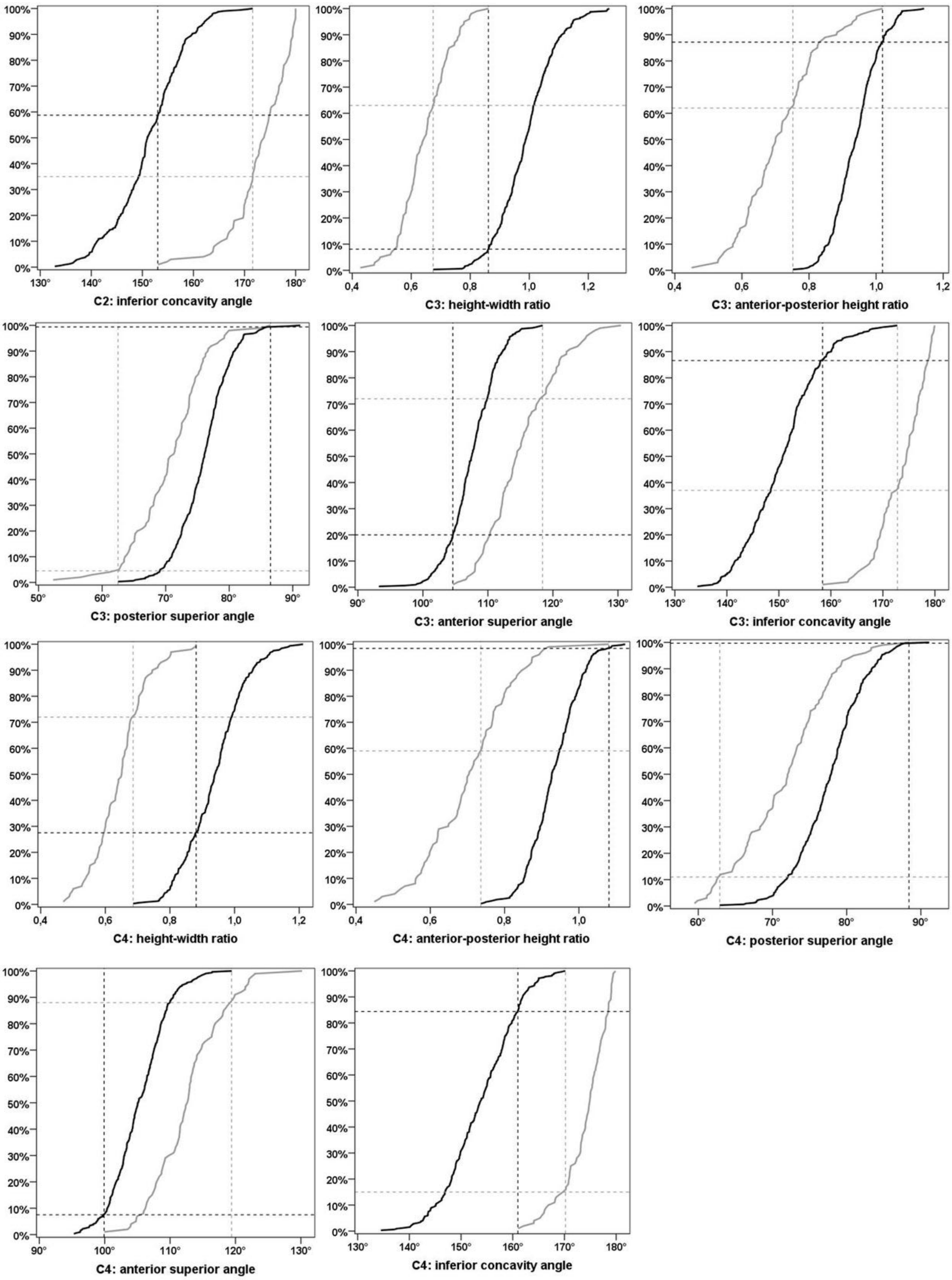
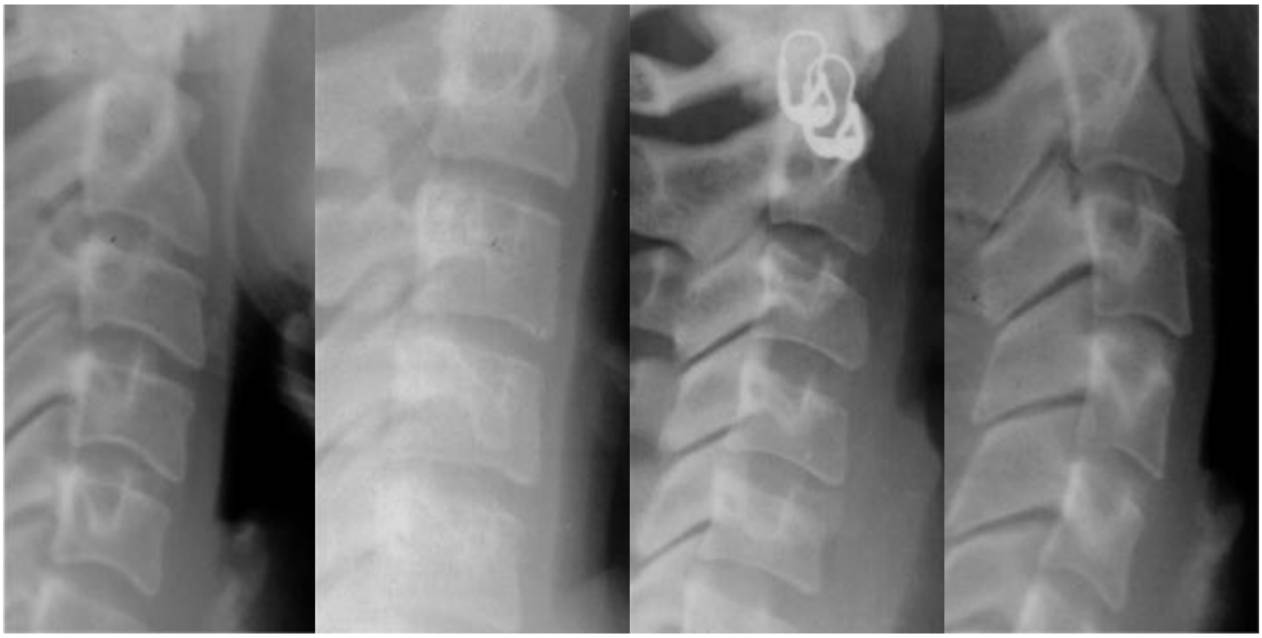


Fig.4: Sample cases of individuals aged 20 years or above illustrating different patterns of violation of the shape criteria for adult cervical vertebrae



Another way of metric evaluation of cervical vertebrae was proposed by Rhee and colleagues.[23] They used cone beam computed tomography which allows for a better exploration of C2, compared to lateral cephalograms. It is hard to conclude from this small exploratory study (35 boys, 45 girls) whether the formulas provide a gain competitive to, or additional to the information available from hand bones. The advantage for forensic age estimation (if there is any) of the additional dose of radiation associated with this approach needs to be demonstrated before it can be routinely used in living subjects.

It is well-known that, due to biological variability, each single method of age estimation is too imprecise in the forensic context. Several methods need to be combined to achieve satisfactory results. The key question is hence which combination of methods is the best one, and not which single method is superior to others. In a recent investigation[24] we have shown how to combine independent age estimates from hand bones and third molars in order to optimally explore the information gathered from each of these well-established methods. We suggest that evaluations of any new methods (using cervical vertebrae or other anatomic structures) should focus on the incremental information gained by the use of these methods on top of those that are routinely applied in forensic age estimation so far. In particular, comparison of dental age estimation methods should not focus solely on the question which of them performs best as a single method. It is rather necessary to examine which one is superior in combination with skeletal age estimation methods. The possibility to get dental and skeletal information from a lateral cephalogram, i.e. a single radiograph, is appealing. However, this combination is probably less promising due to the above studied handicap of the cervical vertebrae.

Nonetheless, we suggest that age estimation from cervical vertebrae should still be studied and be part of the forensic toolkit, even if they were inferior to methods using hand bones. We should have in mind that the hand might not be assessable (for example, if only parts of a body were found), and a second-line age estimation method will be useful in such situations.

CONCLUSION

In comparison to other methods of age estimation, the use of cervical vertebrae suffers from significant handicaps. First, the appearance of adult cervical vertebrae has a large biological variance, and many individuals do not match the descriptions of the "typical" shape that are currently being used. Second, there is a material overlap of the metric ranges of relevant measures between adults and pre-pubertal children which indicates an inferior performance in discriminating different ages. We propose that these handicaps explain the inferior performance of cervical vertebrae in age estimation. We suggest that enhancement of this performance, if possible at all, might require a revised staging of cervical vertebrae development taking into account the variance of metric characteristics described herein. Evaluation of the usefulness of cervical vertebrae for age estimation should demonstrate that their incorporation on top of teeth and hand radiographs provides a gain for the age prediction performance (i.e. more precise age estimates). Odontologists should be aware that forensic age estimation needs to combine dental and skeletal methods and, therefore, knowledge of both should be incorporated in the studies to enhance the precision of age estimation.

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A radiographic study estimating age of mandibular third molars by periodontal ligament visibility

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ABSTRACT

Background: Visibility of the periodontal ligament of mandibular third molars (M3) has been suggested as a method to estimate age.

Aim: To assess the accuracy of this method and compare the visibility of the periodontal ligament in the left M3 with the right M3. The sample was archived panoramic dental radiographs of 163 individuals (75 males, 88 females, age 16-53 years) with mature M3's.

Materials and methods: Reliability was assessed using Kappa. Accuracy was assessed by subtracting chronological age from estimated age for males and females. Stages were cross-tabulated against age stages younger than and at least 18 and 21 years of age. Stages were compared in the left M3 and right M3.

Results: Analysis showed excellent intra-observer reliability. Mean difference between estimated and chronological ages was 7.21 years (SD 5.16) for left M3 and 7.69 (SD 6.08) for right M3 in males and 6.87 (SD 5.83) for left M3 and 8.61 (SD 6.58) for right M3 in females. Minimum ages of stages 0 to 2 were younger than previously reported, despite a small sample of individuals younger than 18. The left and right M3 stage differed in 46% of the 85 individuals with readings from both side and estimated age differed from -10.5 to 12.2 years between left and right.

Conclusion: Accuracy of this method was between 6 and 8 years with an error of 5 to 6 years. The number of individuals with mature M3 apices younger than 18 years was small. The stage of visibility of the periodontal ligament differed between left and right in almost half of our sample with both teeth present. Our findings question the use of this method to estimate age or to discriminate between age younger and at least 18 years.

INTRODUCTION

Olze et al. (2010) described a method of dental age estimation by visualisation of the periodontal ligament of the mandibular third molars (M3) from dental radiographs.[1] Their study was based on a large sample of dental radiographs in Germany aged 15-40 years. The method classifies the visualisation of periodontal ligament width into 4 stage from zero (entirely visible in both roots) to three (visible in only part of one root). This study reported the chronological age for each periodontal ligament visibility (PLV) grade including the minimum age, and suggested PLV was useful to distinguish between individuals younger than and at least 18 and 21 years of age.Another study described the chronological age of these PLV stages in a sample of dental radiographs in Portugal.[2] This second studyreported chronological age for each stage, and noted that the minimum age of PLV stages could not discriminate age at the 18 year threshold but was appropriate for the 21 year threshold in males but not females.

Methods of age estimation need to be tested, and their accuracy and error recorded. [3,4] The aims of this study were to describe the accuracy estimating age by the periodontal ligament width visibility as described by Olze et al. and assess the suitability in assessing the age thresholds of 18 and 21 years. We also compared periodontal ligament width visibility stage in the left and right mandibular third molars if both were present, and assessed the impact of this on estimated age.

MATERIALS AND METHODS

Ethical approval by Queen Mary Ethics of Research Committee number QMERC2016/06.

The archived panoramic radiographic films were of dental patients of mixed ethnic groups attending the Dental Institute. Radiographs were selected by the second author if the mandibular left and/or right third molars (M3) were clearly visible, roots apices mature and teeth fully erupted. Exclusion criteria were incomplete root formation, caries, restorations and impacted/ partially erupted M3's. The sample was radiographs of 163 individuals consisting of 75 males (mean age 23.33, standard deviation (SD) 5.11, minimum 16.76, maximum 45.03) and 88 females (mean age 22.45, SD 5.36, minimum 16.32, maximum 53.94) shown in Figure 1.

Figure 1

The number of M3's assessed on the left side was 128, right side 120 and both left and right M3 were present and included in the study in 85 individuals (total number of M3's 248).

Figure 2

The periodontal ligament of M3's was assessed by the first author on a light box after training and calibration. Each M3 was assigned into one of the four stages of periodontal ligament visibility (PLV) described by Olze et al. (2010).[1] This is illustrated in Figure 2;stage zero is defined as the periodontal ligament visible along the full length of all roots; stage 1 the periodontal ligament is invisible in one root from apex to more than half root; stage 2 is the invisible along almost the full length of one root or along part if the root in two roots or both; stage 3 the periodontal ligament is invisible along almost the full length of two roots.

To assess reliability, 40 teeth were selected from the sample (10 of each stage)and re-examined by the first author and examined by the second author. Intra- and inter-observer reliability were calculated using Cohen's Kappa.

The mean chronological age, SD, minimum and maximum age of each PLV stage was calculated in males and females for left and right side M3.PLV and chronological age were cross tabulated into age stages less than 18, at least 18, less than 21 and at least 21 years of age to assess how well PLV stage discriminated at these age thresholds.

The accuracy of PLV as a method of age estimation was calculated by using the reference mean age of each stage (from Olze et al. 2010) to calculate dental age for left and right M3 in males

Figure 1: Age and sex distribution of radiographic sample of 75 males and 88 females.

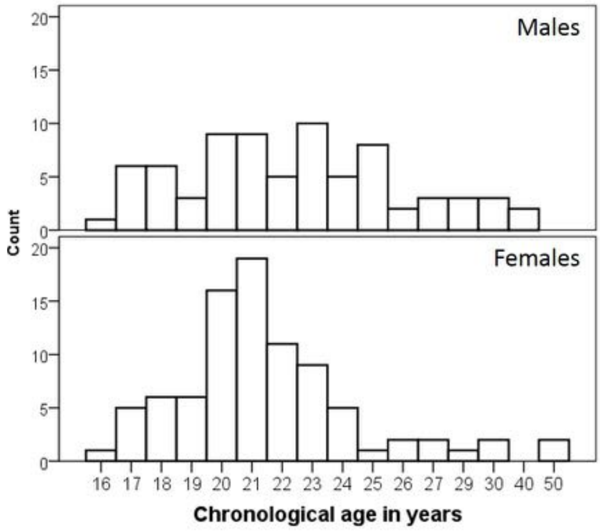
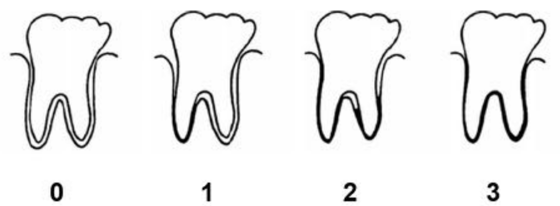


Figure 2: Periodontal ligament visibility stages adapted from Olze et al.[1]



in females.[1] The difference and absolute difference between dental and chronological ages was calculated and a t-test was used to assess the significance of the mean difference between dental and chronological ages.
The PLV stage was compared in the left and right side M3 if both were present in an individual and both were included in the selection criteria (N=85). The percentage agreement and Cohen's Kappa calculated. The difference in dental age between the left and right M3's was calculated for the individuals where PLV stage differed.

RESULTS

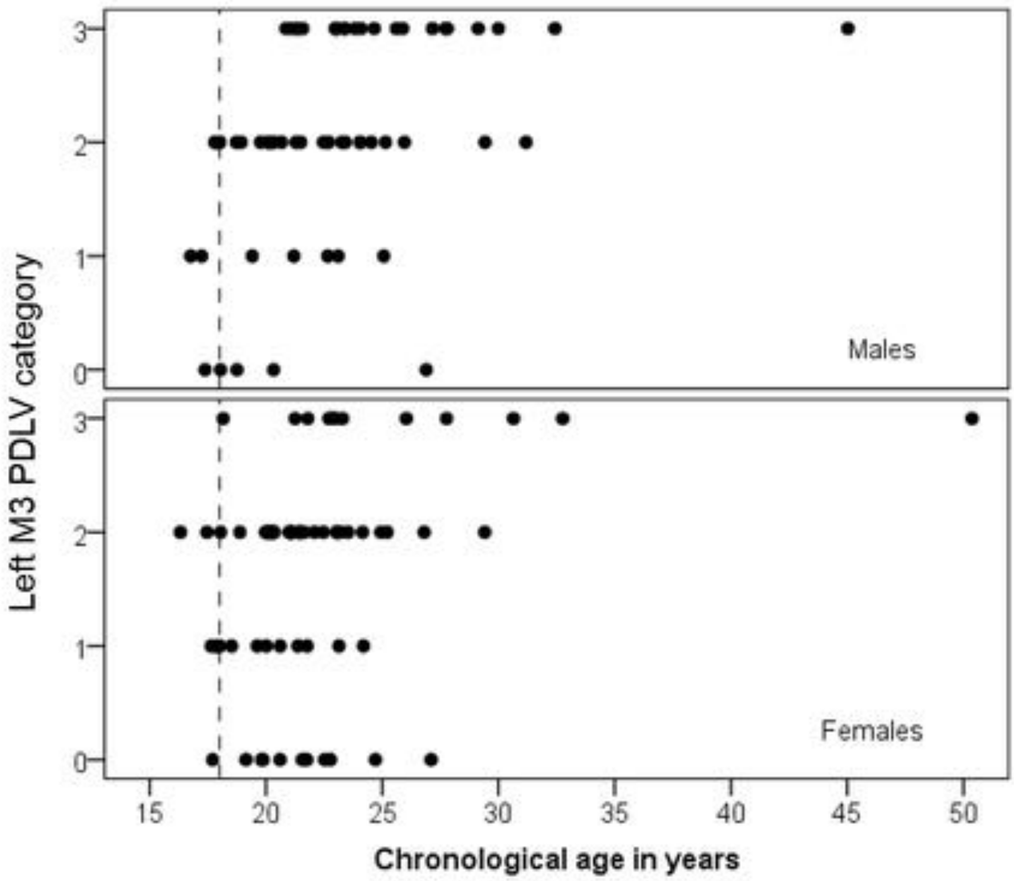
Intra-observer reliability value for Kappa was 0.898 (N=40). Inter-reliability Kappa value was 0.586.

Table 1, Figure 3
Results of mean chronological age, SD, minimum and maximum age of each PLV stage in males and females for left and right side M3 are shown in Table 1. Results of mean age for the left M3 are illustrated in Figure 3 (dashed line is age 18 years). A notable finding is the large age range for all stages including zero (evident prior to 18 up to the late-twenties in this tooth). Most stages included individuals who were younger than 18 years of age. The minimum age of stages 1, 2 and 3 were in some cases younger than the minimum age of stage zero. The age range overlapped considerably between stages.

Table 1: Mean age, standard deviation (SD), minimum and maximum age of PLV category in left M3 and right M3 in males and females

	Side	PLV	N	Mean	SD	Minimum	Maximum
Males	left	0	5	20.28	3.86	17.38	26.89
		1	7	20.77	3.11	16.76	25.06
		2	25	21.98	3.48	17.79	31.18
		3	22	25.70	5.34	20.86	45.03
	right	0	3	22.50	5.85	18.04	29.13
		1	4	18.37	0.89	17.38	19.41
		2	26	23.50	6.87	16.76	45.03
		3	23	24.61	4.32	18.93	34.85
Females	left	0	11	21.59	2.66	17.70	27.10
		1	12	20.05	2.21	17.64	24.19
		2	33	21.56	2.59	16.32	29.41
		3	13	26.42	8.22	18.16	50.37
	right	0	3	18.70	1.10	17.70	19.88
		1	11	19.81	1.33	17.86	21.55
		2	30	22.93	6.31	17.46	53.94
		3	20	23.31	7.03	16.32	50.37

Figure 3: Periodontal ligament visibility stages and chronological age of left mandibular third molar (M3) in males and females. Dotted line is 18 years of age



Tables 2 and 3, Figure 4
Results on the cross tabulation of age thresholds younger than or at least 18 and 21 years of age by PLV are shown in Table 2. No male in stage 3 was younger than 18 years of age (0/22 left M3 and 0/23 right M3). No females presented with left M3 in stage 3 but this differed to the right side where 1/20 was aged younger than 18.
Results of the accuracy of estimating using PLV (Olze et al. 2010)[1] including the mean difference between dental and chronological ages, standard deviation, absolute mean difference, minimum and maximum age are shown in Table 3 (pooled stages) and Table 4 (by PLV stage). The mean difference between dental and chronological ages ranged from 6 to 8 years with a standard deviation of 5 or 6 years. In general terms, the mean difference between dental and chronological ages increased with PLV stage and most comparisons were statistically highly significant.

The results of estimated age (dental age) plotted against chronological ages for males and females are illustrated in Figure 4.The diagonal line shows estimated age equal to chronological age. It is clear that only a few individuals are close to this line and age was over-estimated for most individuals.
Table 5, Figure 5
Results comparing PLV stage of left M3 and right M3 in the 85 individuals with both teeth included in the study are shown in Table 5. Agreement in stage between left M3 and right M3 was evident in 46 out of the 85 individuals (54%). Differences between the left and right M3's (N=39) were evident across all PLV stages. Kappa was 0.33. The difference in estimated age in years when the PLV differed between left and right side plotted against chronological age is illustrated in Figure 5. Estimated age differed as much as -10.5 to 12.2 years.

Table 2: Cross tabulation of PLV category and age thresholds in years

	Side	PLV	N	<18	18+	<21	21+
Males	left	0	5	1	4	4	1
		1	7	2	5	3	4
		2	25	3	22	12	13
		3	22	0	22	1	21
	right	0	3	0	3	2	1
		1	4	2	2	4	0
		2	26	4	22	10	16
		3	23	0	23	6	17
Females	left	0	11	1	10	5	6
		1	12	3	9	8	4
		2	33	2	31	13	20
		3	13	0	13	1	12
	right	0	3	1	2	3	0
		1	11	1	10	8	3
		2	30	2	28	11	19
		3	20	1	19	7	13

Table 3: Mean difference in years between estimated dental age and chronological age

	Side	N	Mean	SD	significance	Absolute mean
Males	left	59	7.21	5.16	0.000	7.91
	right	56	7.69	6.08	0.000	8.93
Females	left	69	6.87	5.83	0.000	7.74
	right	64	8.61	6.58	0.000	9.77

DISCUSSION

The accuracy of PLV as an estimate of age has not previously been reported. The average difference between estimated age from PLV and chronological age ranged from 6 to 8 years with SD values as much as six years. Absolute mean values were from 7 to almost 10 years.

Our results show that there is considerable overlap in age between PLV categories and that there is poor discrimination when age is cross-tabulated into younger than 18 and at least 18 years of age. The minimum ages of PLV stages have been used to exclude individuals as being under 18 or 21 years of age by virtue of their PLV stage. Olze et al. concluded that an individual can be excluded as being under 18 if stages 1, 2 or 3 have been attained and stage 2 can be used to predict the age of 21.[1] Sequeira et al. report that

Figure 4: Scatter plot of dental age versus chronological age in years.Markers show periodontal ligament visibility stages 0 to 3. Diagonal line is dental age equal to chronological age

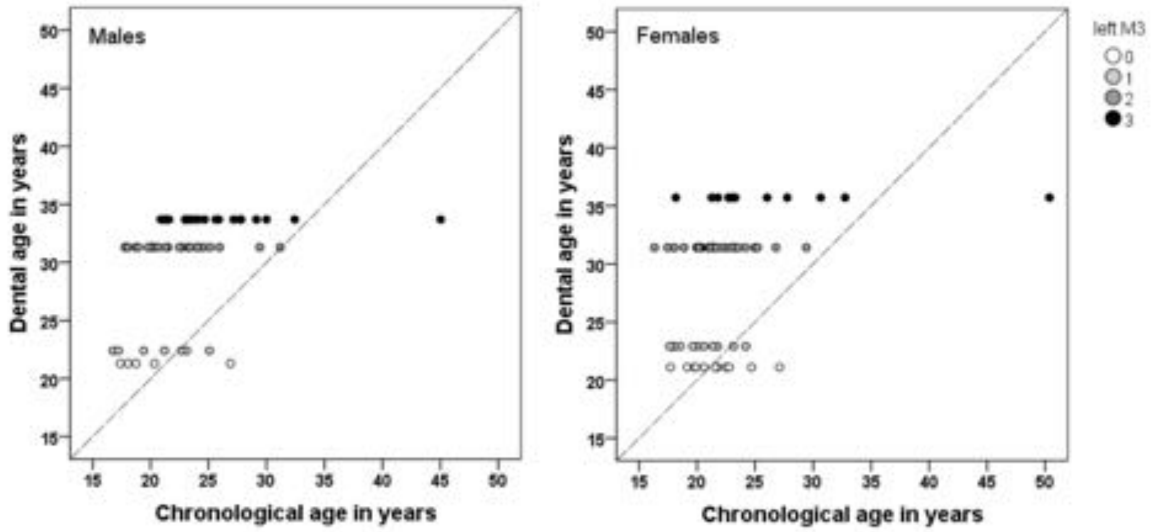


Table 4: Mean difference in years between estimated dental age and chronological ages by periodontal ligament visibility category

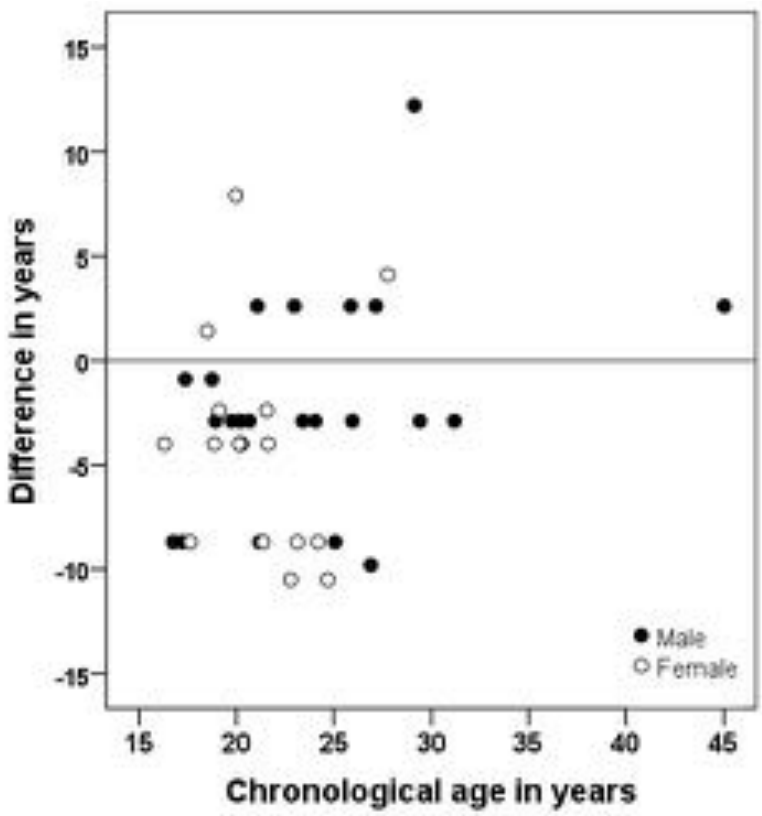
	Side	PLV	Mean	SD	Significance	Absolute	N
Males	left	0	1.02	3.86	NS	3.26	5
		1	1.63	3.11	NS	2.66	7
		2	9.32	3.48	0.000	9.32	25
		3	8.01	5.34	0.000	9.03	22
	right	0	-1.00	5.85	NS	4.09	3
		1	3.83	0.89	0.003	3.83	4
		2	7.60	6.87	0.000	9.64	26
		3	9.58	4.32	0.000	9.64	23
Females	left	0	-0.49	2.66	NS	2.07	11
		1	2.85	2.21	0.001	3.10	12
		2	9.84	2.59	0.000	9.84	33
		3	9.28	8.22	0.002	11.53	13
	right	0	2.80	1.10	0.048	2.80	3
		1	3.69	1.33	0.000	3.69	11
		2	8.67	6.31	0.000	10.16	30
		3	12.09	7.03	0.000	13.59	20

stage 3 rather than stage 2 was a better predictor of the age of 21 years.[2] They report a lower minimum age of stage 2 than stage 1. Lucas et al. also report a lower minimum age of the second stage than the first stage.[5] It is clear from the studies reporting chronological age of PLV stages, that the number of individuals with mature apices of M3 younger than 18 years of age is not large and that the minimum age of PLV stages varies considerably. Our results illustrated in Figures 1 and 3, show that firstly, any sample that includes individuals with mature M3 apices is by its very nature small and secondly, the minimum age of PLV stages was younger than 18 for all stages in females and all but stage 3 in males. These findings demonstrate that the use of the minimum age of PLV stages is inappropriate to include or exclude individuals under 18 or 21 years of age.

Table 5: Comparison of PLV categories of left M3 versus right M3 within the same individual

		Right M3				
		0	**1**	**2**	**3**	**Total**
Left M3	**0**	4	4	3	0	11
	1	3	6	8	0	15
	2	0	1	20	15	36
	3	1	0	6	16	23
	Total	6	11	37	31	85

Figure 5: Accuracy of estimating age from PVL stage of left M3 plotted against right M3 in years. Line is accuracy left M3 equal to right M3



The main inclusion criteria for our radiographic sample was mature apices of M3 and our sample of dental radiographs was small, particularly for individuals with mature M3 apices who were younger than 18 years of age. This lack of individuals younger than 18 with mature M3 root apices is of major importance particularly when PLV is used to estimate the likelihood of being younger than, or at least 18 years of age. None of the studies reporting chronological age of PLV stages[1,2] or modifications thereof[5] detail the *number of individuals* below the age of 18 with mature M3 apices in their samples. The small number of individuals with mature apices younger than 18 has a bearing on the minimum age of PLV stages. Although Olze et al.[1] lists the number of males and females in one-year age stages, they do not report how many of the 17 year olds presented with mature M3 apices. They report a minimum age of stage 0 as 17.6 years in males and 17.2 years in females, suggesting that none of the 15 and 16 year olds presented with mature M3 apices. Sequira et al.[2] state that their inclusion criteria as the presence of mature M3 apices, however, confusingly, their results report the minimum age of M3 stage 0 in males as 18.2. This suggests that either the tables contain a typographical error or none of the 8 males aged 17 years of age had mature apices. Lucas et al.[5] modified the original definitions of PLV stages and assessed a large sample consisting of 100 males and 100 females per year of age from age 16. It can be deduced from their illustration that only 11 out of 200 females and 7 out of 200 males under 18 years of age have mature apices.

Exclusion criteria of any radiographic study are also important and are not always detailed. Two large radiographic studies[1,5] appear to exclude a large proportion of individuals in their total sample (36% and 46% respectively). The reason for this in unclear. Sequira et al.[2] excluded impacted M3 or those with caries or endodontic treatment.

An important finding in our study was the lack of similarity between the left and right side M3 PLV stages (Table 5) and how this impacts on estimated age (Figure 5). A large percentage of individuals differed in PLV stage where both sides were included and this can result in a considerable difference in the estimated age. Few studies compare contra-lateral root shape of third molars from radiographs, however a recent 3D study showed similar M3 root morphology contra-laterally in 81% of a Korean sample i.e. 19% had dissimilar root morphology. [6] A limitation of our study was the small sample and the sequence of scoring. Ideally all left M3's should have been scored and then all right side M3's.

We report results on the accuracy and precision of estimating age using PLV. Reasons for this inaccuracy relates to the anatomy of the periodontal ligament, third molar root variation and difficulties visualising the ligament space. Coolidge [7] measured the periodontal ligament thickness from histological sections summarising findings to show that thickness varied between alveolar crest, mid-root and apex. This paper illustrates some individual results as bar charts and from these raw data, it is possible to calculate average thickness of the periodontal ligament (in hundredths of millimetres) at the alveolar crest in 166 individuals aged 11-16 years was 22.04 (SD 8.59) and in 73 individuals aged 32-50 years was 19.99 (SD 7.29). The mean value in the younger age group is smaller than the older group, however, this is not significantly different. They excluded one outlier, aged 25 with a considerably thinner periodontal ligament width. A study of periodontal ligament thickness from radiographs showed no clear pattern in measurements between erupted, un-erupted, functional or non-functional teeth.[8] The dimensions of the periodontal ligament varied between 259 and 267 microns for erupted M3 and 217 and 341 microns in un-erupted M3, with the maximum thickness seen in impacted M3.[8] The effect of other factors such as the presence of caries, restorations and endodontic treatment on the width of the periodontal ligament is unknown. Third molars with restorations are included in the illustrated examples of stages by Olze et al.[1] and an impacted M3 by Lucas et al.[5]

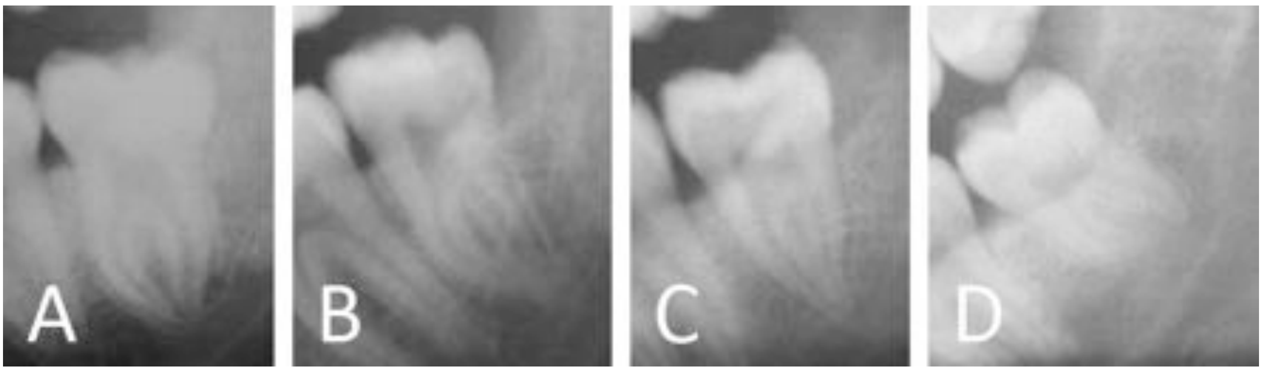
Factors that affect visualisation include the anatomic variation in third molar root morphology and the definition of PLV stages. The anatomy of third molar roots can affect the quality of the radiographic image. The ability of the radiograph to show fine detail (resolution) is measured in line pairs per millimetre and is the ability to discern the boundaries of two objects that are close together. The resolution of a panoramic radiograph is best at the centre of the focal trough but third molar roots may not be favourable placed for high resolution.

Figure 6

Third molars vary considerably in the number, shape and curvature of the roots as well as the separation or fusion of the roots, length of root trunk, bifurcation shape and apical curvature. This is particularly important in three dimensions for endodontics and surgical removal of M3, but also affect the visibility of PLV viewed in two dimensions seen radiographically. The line drawings in Olze et al.[1] depict a typical mandibular molar with two separate roots where the periodontal ligament is clearly visible outlining the mesial and distal root with a clear bifurcation between the roots. Root morphology of M3 is reviewed by Ahmed et al.[9]who reports that the M3 pattern of two separate roots is the most frequent with a prevalence in different studies of 82%[10], 77%[11], 74% [9], 73%[12], 69%[13], 68%[14], 57%[6], 46%[15] and 44%[16]. The roots of M3 can curve towards each other with converging apices with no clear periodontal ligament space (illustrated in Figure 6a and b). If the roots are in close proximity for their entire length, the periodontal ligament space between the roots and at the furcation is not clearly visible (Figure 6c). The prevalence of fused roots of M3 is reported as 19%[14]and 24%.[17]Prevalence of C-shaped roots in cross-section is reported as 11%.[14]The prevalence of the pattern of a single rooted M3 ranges from 56%[16], 52%[15], 42%[6], 24%[13], 21%[12], 17%[11] to 12%[18]. Other factors influencing the clarity of visualising the periodontal ligament of M3includes the proximity of the root apices to the inferior alveolar nerve or the mesial root of M3 being in close proximity to the distal root of the mandibular second molar (see Figure 6b).

Figure 6: Examples of root morphology of M3 where PVL stage is difficult to visualise.

(A) Root apices in close proximity.
(B) Root apices overlapping.
(C) Apical third of roots in close proximity
(D) Mesial root curved and out of focal trough



The PLV stage definitions have limitations. The drawing in Olze et al.[1] suggest that region of unclear PLV extends from the root apex towards the root trunk. In our experience, we noted areas where the PLV was not visible at the bifurcation while the rest of the ligament was visible. We also noted cases where an area of unclear ligament occurred on the lateral surface of a root with clear ligament space around the apex of that and the entire other root. Stage assessment defined by Olze et al.[1] requires training and calibration and the stages are not always easy to identify. Although intra-observer reliability of PLV stage was good, inter-observer reliability was less so, possibly due to the subjective nature of identifying grey levels that distinguish small visual differences that represent the edge of a ligament space adjacent to a curved root surface.[19] Future research on digital radiographs and image analysis software may help to quantify PLV stage.

An attempt to simplify PLV was made by Lucas et al. who adapted the four PLV stages into various percentages of the entire periodontal ligament.[5] Their categories A to D are defined as PLV as 100% visible, 75-50% visible, 50-25% visible and 0% visible respectively. These definitions omit a large proportion of cases (99-76% and 24-1%) and it is unclear how to

assign a third molar with 50% of the periodontal ligament visible is it is both stage B and C.
Results of chronological age of each PLV stage in our study (Figure 3) shows that PLV is a poor maturity marker for several reasons. All PLV stages have a large age range with stage zero observed prior to 18 up to the late-twenties. Most PLV stages were present prior to 18 years of age and the age range overlapped considerably between PLV stages. In addition, the minimum age of PLV stages 1, 2 and 3 were in some cases younger than the minimum age of stage zero. The maximum age of PLV stages is also of interest. Both Olze et al.[1] and Sequira et al.[2] report a maximum age of stage 0 as 30 years (females left M3, males right M3). The considerable age overlap of each of the stages suggests that PLV varies considerably and is not an age related change that can reliably estimate age.
A limitation in our understanding of the rate of PLV is that all studies to date are based on cross-sectional radiographs. Quantifying the rate and severity of age related changes such as PLV of mandibular third molars in the same individual over time is possible from longitudinal radiographs. In the absence of such longitudinal data, we postulate that the nature and rate of increasing PLV stage with age is too variable to be of much practical use to estimate age accurately and is unsuitable to assess if an individual is below or at least 18 or 21 years of age.

CONCLUSION

- Accuracy of PLV stage was between 6 and 8 years with an error of 5 to 6 years.
- The number of individuals with mature M3 apices younger than 18 years was small.
- The stage of visibility of the periodontal ligament differed between left and right in almost half of our sample with both teeth present.
- Our findings question the use of this method to estimate age or to discriminate between age younger and at least 18 years.

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Periodontal Ligament Visibility (PLV): validation of PLV to determine adult status

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periodontal ligament visibility, PLV, dental age estimation

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ABSTRACT

Background: Gradual obliteration of the Periodontal Ligament Visibility (PLV) of lower third molars indicates increasing age. This is used to help determine whether or not an age disputed subject is above or below the 18 year threshold.
Aim: The main focus was to determine, in test subjects of known age, whether the PLV system used 'blind' is able to reliably indicate whether the subject was a child (age <18 years) or adult (age >18).
Materials and methods: A total of 250 normal subjects in the age range 16 to 26 years, from the archives of Guy's Hospital in London, UK, were used to validate the system of PLV. The radiographic assessment of PLV1 was used to categorise four grades of PLV.
Results: It was found that for both females and males PLV-C and PLV-D gave very high probabilities (p = 1.000) of the test subjects being of adult status.
Conclusion: Periodontal Ligament Visibility has the potential to play an important part in the assessment of age disputed asylum seekers who look adult and claim to be children.

INTRODUCTION

Age Assessment using radiographs of the dentition is a reliable way of Forensic Age Estimation. It has been shown that Dental Age (DA) correlates more closely to Chronological Age (CA) than any other Human Biological Growth Marker.[2] This has led to the development of several techniques specific to dental development.
Once the dentition is fully mature it is not appropriate to use the Simple Average Method.[3]
This is because stage H of the Lower Left Third Molar has no further dental development to take place. It has been shown that an accurate estimate of the summary statistics for stage H in a UK Caucasian sample leads to a minimum value of 15.47 years, a mean or median value of approximately 19.40 and a maximum value of 21.64 Years.[4] It has been shown that using the summary statistics for Stage H alone the probability that a subject is over 18 years old is of the order of 90%.[5] A more realistic estimate, following appropriate censoring of the data for Stage H, is a probability that the subject is over 18 years of only 79.6%.[3] Thus using the technique of probability estimates there is an 80% chance that a subject with stage H is older than 18.00 years. The corollary to this is that there is a 20.4% chance that the subject is younger than 18 years. There are similar data from Austria.[6] The conclusion from this Austrian

article was that "As a single criterion for age estimation, wisdom teeth are not suitable, especially regarding the question of attained age of 18 years old."

One method suggested to overcome this problem is to use the Periodontal Ligament Visibility (PLV) as discernible on a Dental Panoramic Radiograph (DPT).[7] This Growth Marker extends the age range of summary statistics to over 30 years old. This fills a gap between 18 years when tooth development declines in importance and the older growth markers of stages of development of the Sterno Clavicular Joint.[8]

The principles of the work on third molars conducted in Germany was repeated in London, UK, with a balanced study on subjects between 16 years and 26 years.[7] A team in Portugal,[9] although using a slightly different age range, gave broadly similar results to the German Team.

The present study was conducted to test the validity of the data from this large study on Periodontal Ligament Visibility[7] when applied to patients of known age, gender and ethnicity, assessed 'blind', and drawn from the clinical archives of Guy's and St Thomas' Hospital in central London.

MATERIALS AND METHODS

In the UK the use of patient databases does not require Research Ethics Approval. The approach is to regard the project as an audit project. This was approved by the Lead Clinician in Orthodontics at Guy's and St Thomas' NHS Trust.

A consecutive sample of patients' records at Guy's Dental Hospital from January 2015 to March 2015 was used as the preliminary sample. Only patients for whom a Dental Panoramic Tomograph was available were isolated from the patients records archive for study. An age range filter was applied so that all subjects were aged between 16.00 years and 25.99 years. All patients with DPTs were recruited on a consecutive basis.

A Microsoft Excel spreadsheet was created to enable entry for the Demirjian Tooth Development Stages (TDS), and also for the gender, date of birth and date of radiograph. For each subject with a Dental Panoramic Tomograph (DPT) an assessment of the Lower Third Molar (LL8) was made to determine if it was mature (Demirjian Stage H).[10]

Reliability of the assessments of the TDS was performed by randomly selecting 100 cases of the subjects and re-assessing them 1 month later. This process was repeated for the reliability of the assessments of the categories for the Periodontal Ligament Visibility. Only Within Observer Agreement was explored as only one investigator (VSL) carried out the assessments for this validation study.

For subjects with a mature Stage H the appearance of the Periodontal Ligament of LL8H was then assessed (Figure 1) and entered into the Excel spreadsheet.

The assessments were performed with the age and gender of the subjects masked from the observer. The age of the subjects was then calculated using the date of radiograph and subtracting from this the date of birth. This was converted to decimal years to give the age of each subject.

These assessments were then subjected to a filter process in the Excel spreadsheet. First those subjects with a TDS other than Stage H were removed. Second, the subjects with PLV-A were then filtered according to the PLV Stage. First for PLV-A and then repeated for PLV-B, PLV-C and PLV-D. The assignment of below or above the 18 year threshold was made on the basis of the data in the original publication which is reproduced below (Table 1).[7]

RESULTS

A total of 250 subjects were recruited to the study. This comprised 145 females and 93 males (Table 2). Information from the Clinical Records was incomplete for 12 patients.

A proportion of these, approximately 7.5% overall, were unsuitable. This was by virtue of the DPT exhibiting Stage F or G or the images of the molar teeth being of poor quality. To enable appropriate utilization of the PLV characteristics it is essential that Demirjian Stage H is present in the subject or person for whom the Threshold Assignment is required.

The Within Rater Assessments for reproducibility was 97% [Kappa Value 0.9281] for the Tooth Development Stages, and 94.12% [Kappa Value 0.894] for the Periodontal Ligament Visibility categories. These values fall within the highest range i.e. Very Good.[11]

Although the Reference Data Set gives outcomes for PLV-A and PLV-B with a small number of subjects under 18 Years (less than 10%,)[7] in the present study, there are no subjects with PLV-A or PLV-B for females or males who were likely or possibly under 18 years old.

Figure 1: Schematic drawings and radiographic examples of Periodontal Ligament Visibility (reproduced from the Open Access article Lucas et al. 2017).[7]

PLV – A = 100% to 74% of the periodontal ligament around the lower left third molar is discernible on the DPT.
PLV – B = 75% to 50% of the periodontal ligament is visible.
PLV – C = 50% to 25% of the periodontal ligament of the lower left third molar is visible when summated across the mesial and distal roots.
PLV – D = 25% to 0% of the periodontal ligament is discernible.

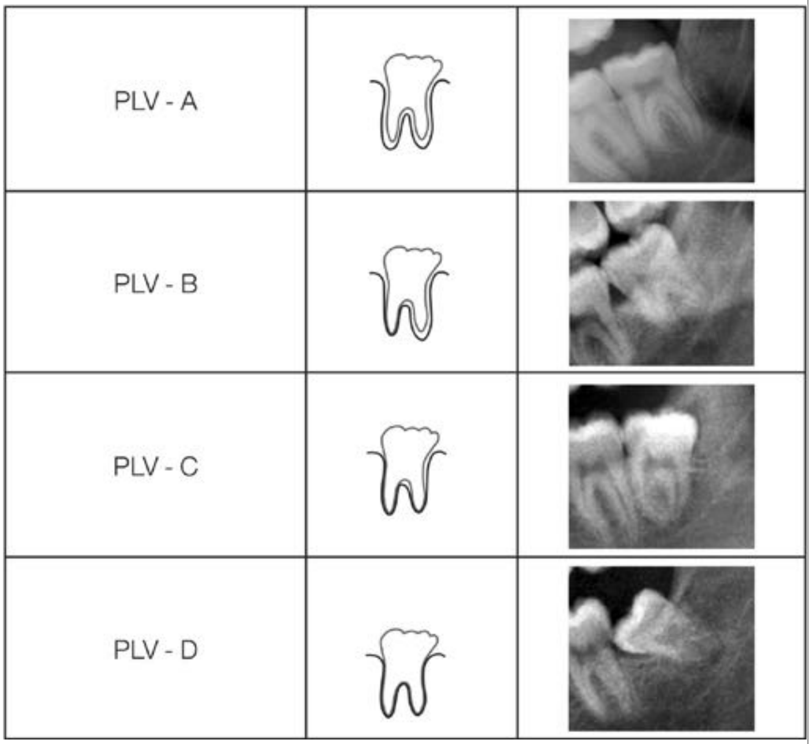


Table 1: Reproduced reference data[7] used to assign the subjects of this validation study to an appropriate threshold status i.e. below or above 18 years old

	n-tds	**$\bar{x}$-tds**	**sd-tds**	**min-tds 0th%ile**	**25th%ile**	**median 50th%ile**	**75th%ile**	**max-tds 100th %ile**	**Younger than 18 Years**	**p >18 Years**
FEMALES										
PLV-Af	8	19.57	1.83	16.33	18.23	20.28	20.60	22.06	14.20%	0.858
PLV-Bf	202	21.25	2.16	16.17	19.80	21.21	22.61	25.83	5.90%	0.941
PLV-Cf	277	22.96	1.95	18.08	21.43	23.36	24.47	25.95	**0.00%**	**1.000**
PLV-Df	54	23.86	1.79	18.58	22.66	24.33	25.39	25.99	**0.00%**	**1.000**
MALES										
PLV-Am	12	20.32	1.61	17.69	19.58	20.27	21.48	22.80	9.00%	0.910
PLV-Bm	151	21.17	2.13	17.62	19.48	20.85	22.68	25.43	2.60%	0.974
PLV-Cm	308	22.49	2.11	18.10	20.86	22.63	24.22	25.43	**0.00%**	**1.000**
PLV-Dm	87	23.37	1.85	18.67	22.29	23.61	24.94	25.93	**0.00%**	**1.000**

Table 2: Age distribution of subjects recruited from Clinical Records Database

Age Range (Years)		Females	Males	All Subjects
16.00 to 16.99		6	11	17
17.00 to 17.99		2	9	11
18.00 to 18.99		10	8	18
19.00 to 19.99		11	6	17
20.00 to 20.99		9	7	16
21.00 to 21.99		16	9	25
22.00 to 22.99		15	11	26
23.00 to 23.99		27	14	41
24.00 to 24.99		28	7	35
25.00 to 25.99		21	11	32
Clinical Data Absent				12
TOTAL		145	93	250

The Within Rater Assessments for reproducibility was 97% [Kappa Value 0.9281] for the Tooth Development Stages, and 94.12% [Kappa Value 0.894] for the Periodontal Ligament Visibility categories. These values fall within the highest range i.e. Very Good.[11]
Although the Reference Data Set gives outcomes for PLV-A and PLV-B with a small number of subjects under 18 Years (less than 10%,)[7] in the present study, there are no subjects with PLV-A or PLV-B for females or males who were likely or possibly under 18 years old.
The data presented in Table 3 are taken from the radiographs in the Guy's Hospital Archives. They are all subjects whose Chronological Age is Greater than 16.00 Years. And up to 26 years old apart from one case of 26.03 years old. The information relates to males in the top half of Table 2, and females in the lower half. The subjects for whom the PLV assessment can be utilised all exhibited Stage H of the Lower Left Third Molar.
The data are focused on the utility of PLV as an indicator of the Probability or Likelihood that a male or female subject with a Third Molar at stage H is older than 18 Years. Subjects with PLV-suffix were not suitable for MMM assessment.
There are a number of subjects when the one or more of the markers was not useable. These figures are given in b II, c II, h II, and i-II.
In all subjects the Threshold Assignment Method (TAM) gave a high %age probability of the subject being over 18 years.

DISCUSSION

The data presented her show that in a validation study the RPV of a consecutive sample of normal dental hospital patients who exhibit Stage H there is a very high number of estimates in the 250 consecutive subjects who are assigned an age over 18 years. The age range 16.00 to 25.99 years was used as this covers the age range when the Lower Left Third Molar may first acquire stage H and ensures that all subjects in the sample have attained maturity. Using the criterion of PLV when Demirjian Stage H is present effectively gives a very high confidence in assigning a subject to 'Over 18 Years old'. It is clear that for a small number of our 250 validation subjects, at least in

Table 3: Periodontal Ligament Visibility - Validation Assessment of the proportion of subjects correctly assigned to over 18 years from a consecutive convenience sample of subjects with known ancestry, known gender, and known age. The probability (P) of a subject being over 18 years is given as a proportion of 1, and also as a percentage equivalent. The letters 'a' through to 'l' indicate the rows of the table, the Roman numerals indicate the columns. The lower case letter a to l and the Roman numerals I to VI are used for easy location of the cells within the table. For instance, d II is the cell with 22/87 ie 22 of the 87 male cases exhibiting Stage H also exhibited PLV-B in the data.

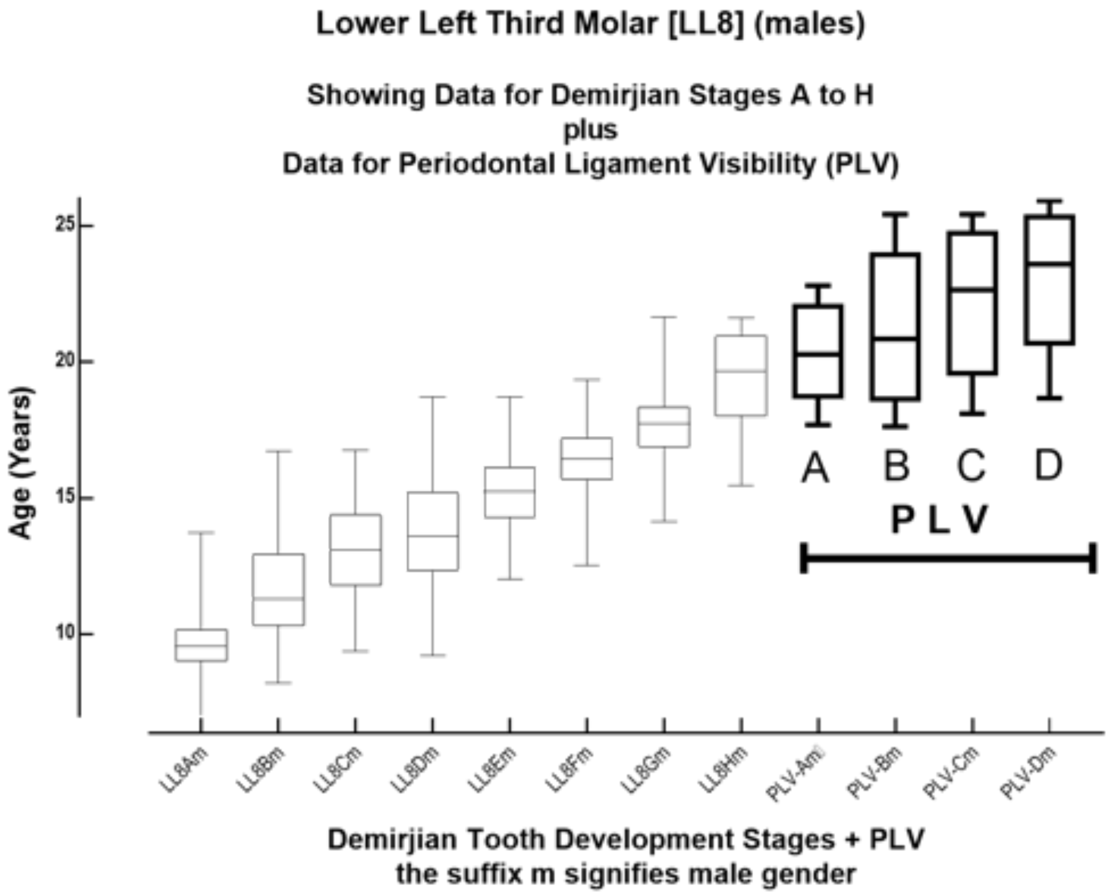
	I	II	III	IV	V	VI
a	**Males m**	**n**	**%age of Sample**	**Age Range in Years**	**P > 18 Years**	**%age Probability > 18**
b	PLV - •	0/87	0	na	na	na
c	PLV - Am	0/87	0	na	na	na
d	PLV - Bm	22/87	25.28	19.31 to 29.65	1.000	100.0
e	RPV - Cm	53/87	60.92	18.04 to 26.04	1.000	100.0
f	RPV - Dm	10/87	11.49	19.89 to 26.03	1.000	100.0
g	**Females f**	**n**	**%age of Sample**	**Age Range in Years**	**P > 18 Years**	**%age Probability > 18**
h	PLV - •	26/163	15.95	na	na	na
i	PLV - Af	0/163	0	na	na	na
j	PLV - Bf	37/163	22.70	18.55 to 25.89	1.000	100.0
k	RPV - Cf	85/163	52.15	21.88 to 25.93	1.000	100.0
l	RPV - Df	15/163	9.20	20.97 to 26.03	1.000	100.0

females, it is not possible to use PLV as a criterion. This was because the root development was at stage F or G and therefore RPV could not be assessed. The use of this criterion needs to be investigated by other research workers to determine whether or not there is wider applicability of this developmental or bony marker in other ancestral groups.

At present it is not clear as to the biological changes occurring that are responsible for the diminishing periodontal ligament visibility with increasing age. A preliminary assessment with Cone Beam Computed Tomography suggest that there is increasing bony deposition, both cortical and medullary, in the mandibular bone buccal to the lower third molars. It is emphasised that this is a preliminary assessment which requires confirmation by an appropriately designed study.

A fortuitous outcome is the way the summary percentile data for PLV extend the data for Demirjian from approximately 18 years through to 26 years. In terms of providing supporting evidence of the subject being over 18 years this information is compelling. It is helpful to see this plotted out in a simple graph (Figure 2).

Figure 2: Box and whisker plots of Demirjian Tooth Development Stages from stage A through to H. Appended at the right (older) side of the graph are the box and whisker plots for Periodontal Ligament Visibility categories



The four PLV stages form a continuum from the median value for the Demirjian tooth development stages from LL8A through LL8Hm and on through PLV stages to the values for the Sterno Clavicular Joint (SCJ) stages 4 and 5.[8]
The graph shows the box and whisker plots for LL8Am through to LL8Hm where the suffix m indicates male gender. The data for the box and whisker plots is from the UK Caucasian Reference Data Set accessible in www.dentalage.co.uk/+R
Further work is planned to extend the age range of subjects to explore the impact of PLV on the dental age assessment in different ancestral groups.
It is important to note that the use of PLV requires only a single Dental Panoramic Radiograph.

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A radiographic study of the mandibular third molar root development in different ethnic groups

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ABSTRACT

Background: The nature of differences in the timing of tooth formation between ethnic groups is important when estimating age.
Aim: To calculate age of transition of the mandibular third (M3) molar tooth stages from archived dental radiographs from sub-Saharan Africa, Malaysia, Japan and two groups from London UK (Whites and Bangladeshi).
Materials and methods: The number of radiographs was 4555 (2028 males, 2527 females) with an age range 10-25 years. The left M3 was staged into Moorrees stages. A probit model was fitted to calculate mean ages for transitions between stages for males and females and each ethnic group separately. The estimated age distributions given each M3 stage was calculated. To assess differences in timing of M3 between ethnic groups, three models were proposed: a separate model for each ethnic group, a joint model and a third model combining some aspects across groups. The best model fit was tested using Bayesian and Akaikes information criteria (BIC and AIC) and log likelihood ratio test.
Results: Differences in mean ages of M3 root stages were found between ethnic groups, however all groups showed large standard deviation values. The AIC and log likelihood ratio test indicated that a separate model for each ethnic group was best. Small differences were also noted between timing of M3 between males and females, with the exception of the Malaysian group. These findings suggests that features of a reference data set (wide age range and uniform age distribution) and a Bayesian statistical approach are more important than population specific convenience samples to estimate age of an individual using M3.
Conclusion: Some group differences were evident in M3 timing, however, this has some impact on the confidence interval of estimated age in females and little impact in males because of the large variation in age.

INTRODUCTION

Developing teeth are frequently used to estimate age and a number of methods are available (see review by Liversidge).[1] Most of these divide the growth and development of a tooth into discrete crown and root stages. Once a tooth formation stage has been identified, dental age can be calculated from a selected reference data.
Studies show that females on average, are earlier in dental development of most teeth with the exception of root stages of

the third molar[2-4] and this means that sex-specific reference data are appropriate.[1] Many studies report differences in the timing of tooth development in various parts of the world and these have been interpreted as regional and ethnic differences,[5-17] although this has been questioned because of differences in sample structure, sample size and most importantly, statistical analyses.[18-20] Newer approaches to calculating the probability of age, given a tooth stage are considered more appropriate than mean age of individuals within a tooth stage.[21-24]

Recommendation from the Study Group on Forensic Age Diagnostics for age estimation in the living[25] state that data on the reference population regarding genetic/geographic origin should be provided, as it is still unclear if ancestry/region impact on estimated age. The aims of this study were (1) to compare regional and sex differences in root development of the mandibular third molar (M3) for given ages; (2) to derive age distributions for given stages of root development of M3; and (3) to consider the possibility of an individual being at least/older than 18 years at given stages of root development of M3.

MATERIALS AND METHODS

Data were collected from archived dental radiographs of patients attending dental schools or private clinics during 2003-2015. The sample consisted of panoramic or lateral radiographs of 4555 individuals aged 10-25 years. Inclusion criteria were clear image of M2 and M3, recorded date of birth and date of radiograph. Chronological age was calculated as the time from date of birth to the date of radiographs. In 29% of cases from Nigeria, South Africa, and Senegal, only year of birth was available. In these cases, age was assumed to be half way into their year i.e. if the age was 14 years then age was assumed to be 14.5 years old. Health status and socio-economic status were not recorded.

The following regional groups were considered:

1. Sub-Saharan African (Nigeria, Senegal and South Africa)
2. Japanese
3. Malaysian
4. White/European UK
5. Bangladeshi UK

The age distribution for the different regional groups for male and females is shown in Table 1. The development of mandibular left third molar (M3) was scored by the first author based on Moorrees et al.[2] (illustrated in Figure 1) following descriptive criteria.[26] Tooth stages of M3 in the Malaysian radiographs were scored by the second author and inter-observer reliability was calculated from 20 radiographs (Kappa 0.81). Data from South Africa and UK groups have been analysed in previous reports of M3 development and age estimation. [26-28]

Figure 1: Molar crown and root stages after Moorrees. (Ri = initial root, Rcl = root cleft, R¼ = root quarter, R½ = root half, R¾ = root three quarters, Rc = root complete, A½ = apex half closed, Ac = apex closed)

Table 1: The age and sex distribution from the different regional groups, 2028 males, 2527 females. (Age in years, M = male, F = female)

Age	Regional group									
	Sub-Saharan African		Japanese		Malaysian		White/UK		Bangladeshi/ UK	
	M	F	M	F	M	F	M	F	M	F
10	69	87	53	57	42	29	42	35	27	23
11	71	85	49	52	38	60	38	47	19	13
12	84	77	27	30	62	63	41	34	21	16
13	69	65	12	21	49	68	35	34	23	24
14	51	68	14	22	40	63	26	46	23	11
15	54	52	14	23	41	71	28	34	33	31
16	29	43	9	25	29	64	28	39	29	14
17	33	39	7	26	23	36	27	51	21	20
18	37	42	9	18	13	27	35	42	20	29
19	33	35	5	10	17	24	29	62	13	30
20	32	27	6	16	14	15	35	52	15	35
21	27	20	7	13	17	12	22	57	17	7
22	24	9	2	9	16	17	19	36	18	21
23	13	18	0	8	1	3	9	19	19	8
24	13	34	5	2	0	0	8	10	17	10
25	14	20	0	5	0	0	8	7	9	4
Total	653	721	219	337	402	552	430	605	324	310

We have taken a transition analysis approach (as described in Boldsen et al.)[29] that is a parametric method for "modelling the passage of individuals from a given developmental stage to the next higher stage in an ordered sequence." [18] We assumed a distributional form for the transitions between maturity stages, and fitted this model by maximum likelihood estimation.[18] We then derived the age distribution conditional on stage through a Bayesian estimation procedure (further information in supplementary methods). Hence, this is two-step modelling approach; 1) fit a probit model for estimating the mean age of transition between the stages and the estimated common standard deviation for transitions (on log scale) and 2) use the estimates from step 1 to derive the age distributions for each stage.

In the second step in the age estimation procedure, we considered the estimates obtained from the probit model obtained in step one as given, without considering estimate uncertainty in step one. This was done for simplicity, reducing the complexity of step two. Hence, the estimated age distributions given molar stage represent the age distribution from the fitted probit model, given that the estimated mean age of transition between the stages and the estimated common standard deviation for transitions is the most likely age distribution. This does not take account of the uncertainty in the fitted probit model and this might increase the size of the uncertainty intervals for age, a point that warrants further investigation.

For an introduction in the Bayesian way of thinking in biological anthropology, see Konigsberg and Frankenberg.[20] Taking this Bayesian approach in the last step we obtained 2500 age samples for each molar and stage. The Bayesian equivalence of a confidence interval is a credibility interval, and a 95% interval is given by the 2.5 and 97.5 percentiles in the distribution of the 2500 samples. Further statistical information is provided in Supplemental Methods.

RESULTS

The distribution of tooth formation stages of M3 and chronological age for males and females is shown in the scatterplots in Figures 2 and 3. Only tooth stages that begin after age 10 (the minimum age of our sample) are included in these illustrations (root three quarters to apex closed).

Figure 2: Third molar root stage versus chronological age in years in males. Stages see Figure 1

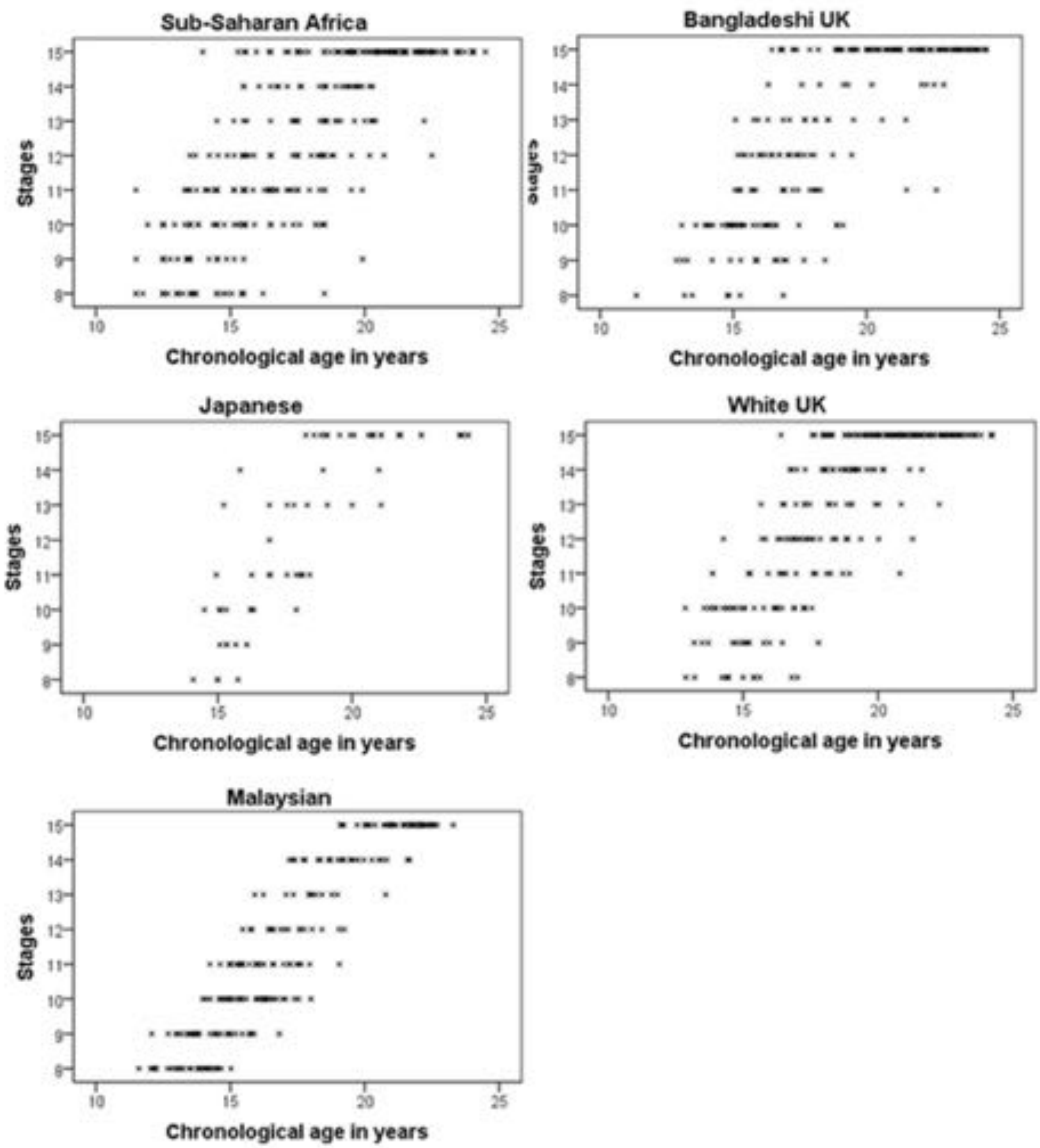
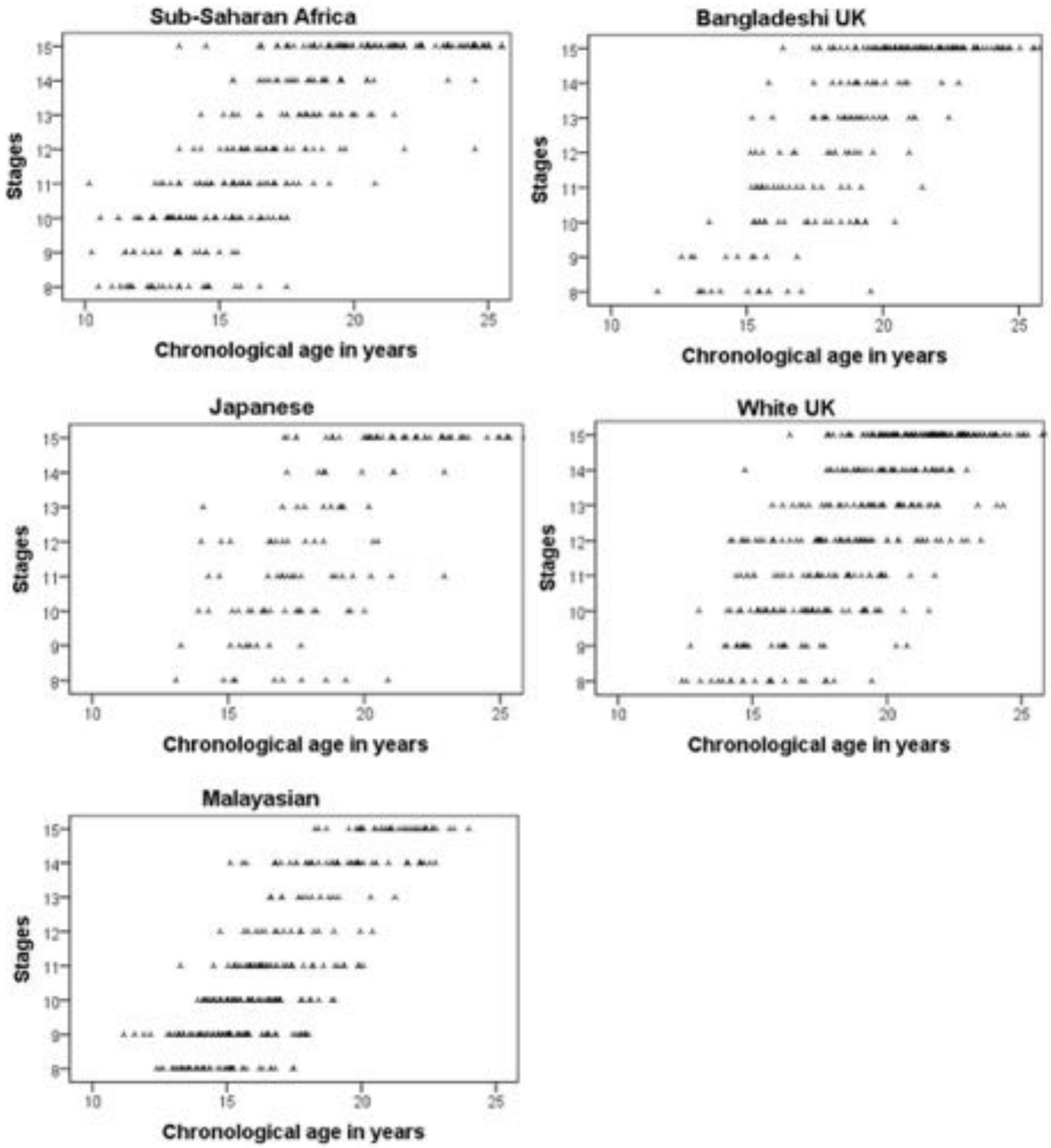


Figure 3: Third molar root stage versus chronological age in years in females. Stages see Figure 1

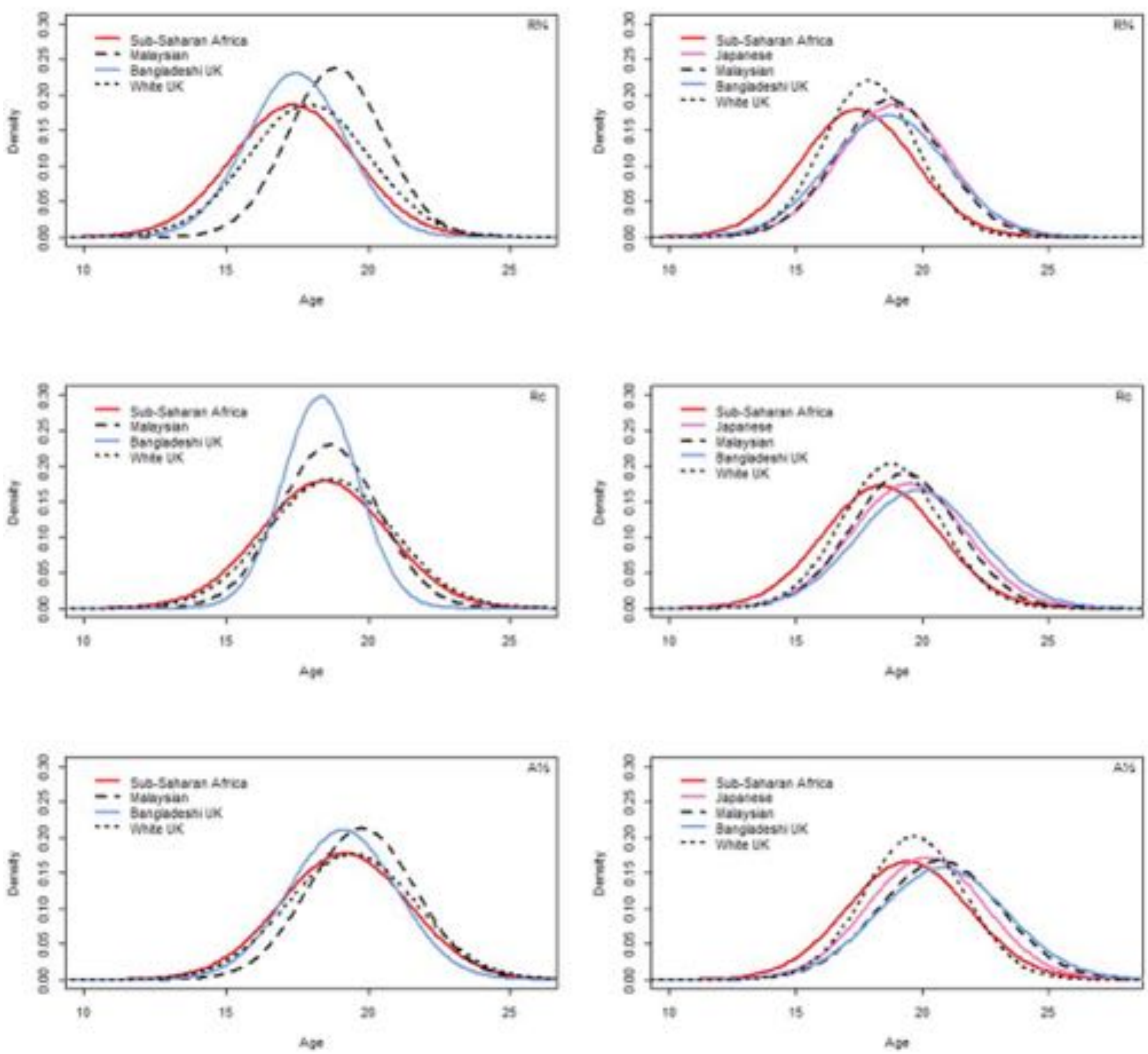


These figures show that each stage occurs over a wide age range and that each age group has a wide variation in scores. From Figure 2 we can see that there are relatively few individuals younger than 15 years in Japanese males and consequently this regional group was not included in further analyses. The plots demonstrate a greater variation in tooth stages in females than in males for all regional groups. Malaysian, both males and females, seem to show least age variation within each stage.

The estimated age density plots for some late root stages of M3 (stages R¾ to $A_{1/2}$) are shown in Figure 4.

The mean age for M3 stages from initial root (Ri) to apex half closed (A½), standard deviation (SD)

Figure 4: Estimated age density plots for M3 stages in males (left) and females (right). (R¾ = root three quarters, Rc = root complete, A½ = apex half closed)



and 95% credibility interval (95% CI) for each ethnic group are shown in Table 3. These estimates of mean age, given a root stage, show very little variation between males in different regional groups. The SD varies between 1.33 for the Malaysian males (stage Ri) and increase with stages of root growth to 2.26 years for Bangladeshi UK males (stage A½). All groups have a mean age older than 18 years for stage Rc, but 18 years could not be excluded from any of the groups (R_i- $A_{1/2}$) with 95% confidence.

Table 3 shows the estimated and observed percentage of individuals 18 years for the stages from initial root formation to apex half closed. This demonstrates that 68% to 82% of individuals are older than 18 years when the root development in M3 is in stage A½. The females are slightly later in development and 71% to 88 % were older than 18 years at stage A½.

The results comparing three model alternatives for M3 are shown in Table 4 for ethnic group comparisons for males and females. Interpreting BIC criteria, AIC and the log likelihood ratio test show some differences. The best model using BIC is the joint model (lowest BIC value) for both males and females. The best model using AIC suggest that a separate mode for each ethnic group l is best for both males and females (lowest AIC value). The log likelihood ratio test indicates that a separate model for each ethnic group.

Table 2: Estimated mean age, standard deviation (SD) and 95% credibility interval (95% CI) for the mandibular left third molar (M3) in years. (Ri = initial root, Rcl = root cleft, R¼ = root quarter, R½ = root half, R¾ = root three quarters, Rc = root complete, A½ = apex half closed)

Group		**Males**			**Females**		
	Stage	**Mean**	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**
Sub-Saharan Africa	Ri	14.13	1.70	11.11, 17.74	13.71	1.74	10.59, 17.51
	Rcl	14.58	1.80	11.44, 18.39	14.18	1.76	11.06, 17.91
	R¼	15.26	1.86	11.95, 19.25	15.04	1.96	11.62, 19.25
	R½	16.46	2.00	12.81, 20.61	16.40	2.10	12.66, 20.88
	R¾	17.36	2.15	13.39, 21.89	17.43	2.23	13.41, 22.00
	Rc	18.43	2.23	14.37, 23.18	18.38	2.32	14.43, 23.29
	A½	19.16	2.25	15.17, 23.98	19.42	2.41	15.02, 24.67
Japanese	Ri				16.06	1.84	12.78, 20.11
	Rcl				16.52	1.86	13.19, 20.49
	R¼				17.16	1.97	13.72, 21.43
	R½				18.01	1.99	14.43, 22.20
	R¾				18.85	2.15	15.09, 23.39
	Rc				19.46	2.28	15.34, 24.18
	A½				20.15	2.33	16.01, 25.04
Malaysian	Ri	14.10	1.33	11.74, 16.92	14.51	1.60	11.62, 17.88
	Rcl	14.92	1.43	12.37, 17.90	15.52	1.73	12.46, 19.16
	R¼	15.88	1.50	13.10, 19.10	16.66	1.86	13.19, 20.64
	R½	16.89	1.64	13.91, 20.34	17.79	1.99	14.39, 22.12
	R¾	17.85	1.68	14.82, 21.52	18.70	2.07	14.95, 23.10
	Rc	18.62	1.74	15.46, 22.27	19.29	2.10	15.44, 23.75
	A½	19.76	1.88	16.36, 23.65	20.68	2.39	16.41, 25.73
White UK	Ri	14.66	1.44	12.04, 17.64	15.07	1.88	11.65, 19.15
	Rcl	15.12	1.47	12.37, 18.33	15.64	1.86	12.26, 19.52
	R¼	15.86	1.60	12.93, 19.04	16.66	2.04	12.99, 21.00
	R½	16.65	1.66	13.67, 19.93	17.63	2.15	13.77, 22.17
	R¾	17.45	1.73	14.29, 20.95	18.63	2.34	14.35, 23.66
	Rc	18.32	1.84	15.04, 22.29	19.82	2.40	15.57, 24.95

	A½	19.10	1.90	15.62, 23.08	20.79	2.54	16.24, 26.12
Bangladeshi UK	Ri	14.16	1.68	11.12, 17.70	14.84	1.58	11.96, 18.21
	Rcl	14.72	1.74	11.54, 18.39	15.42	1.66	12.43, 18.87
	R¼	15.86	1.90	12.53, 19.94	16.24	1.67	13.13, 19.71
	R½	17.00	1.98	13.57, 21.25	17.14	1.81	13.96, 20.91
	R¾	17.90	2.15	14.04, 22.43	17.89	1.82	14.68, 21.63
	Rc	18.67	2.20	14.67, 23.46	18.75	1.97	15.17, 22.80
	A½	19.42	2.26	15.45, 24.20	19.68	1.99	15.99, 23.77

Table 3: The estimated and actual observed (in parenthesis) percent of individuals 18 or older for M3 stages R¼ to A½. (R¼ = root quarter, R½ = root half, R¾ = root three quarters, Rc = root complete, A½ = apex half closed)

M3 stage	**Males**				**Females**				
	Sub-Saharan Africa	**Malaysia**	**White UK**	**Bangladeshi UK**	**Sub-Saharan Africa**	**Malaysia**	**Japan**	**White UK**	**Bangladeshi UK**
R¼	8.44 (10.87)	8.68 (2.94)	8.88 (0.00)	13.44 (8.82)	7.84 (0.00)	22.08 (8.47)	31.56 (23.81)	24.00 (25.00)	14.60 (40.91)
R½	21.68 (10.20)	24.56 (3.45)	20.48 (33.33)	28.84 (31.58)	20.60 (5.88)	42.36 (26.09)	49.68 (41.18)	40.80 (48.65)	30.44 (22.22)
R¾	36.00 (34.29)	44.16 (23.53)	36.32 (21.14)	45.68 (14.29)	37.44 (21.62)	61.40 (29.41)	62.68 (30.77)	59.00 (57.89)	45.44 (56.25)
Rc	55.72 (58.62)	62.08 (50.00)	56.16 (55.56)	60.40 (46.67)	54.52 (60.00)	72.12 (50.00)	72.40 (60.00)	77.56 (78.26)	62.12 (72.00)
A½	68.04 (62.50)	82.96 (80.00)	71.16 (82.76)	72.80 (80.00)	71.36 (62.86)	88.12 (72.09)	82.60 (88.89)	87.12 (89.36)	80.04 (88.89)

Although there is some disparity depending on which test is used, this suggests that there are significant differences between ethnic groups in the timing of M3 in both males and females.

The results comparing three model alternatives for M3 are shown in Table 5 for male female comparisons for each ethnic group. Small differences are apparent between the BIC and AIC approaches, indicating that there are small but significant differences between males and females for all groups, with the exception of Malaysians. These differences between the timing of M3 formation in males and females are smaller than those between ethnic groups.

Table 4: Comparison of three model alternatives testing M3 formation in ethnic groups: (1) a separate model for each ethnic group, (2) a joint model for all ethnic groups, same α and β (3) a joint model for all ethnic groups, same α but different β. (Log L = log likelihood, n = number of parameters, BIC = Bayesian information criteria, AIC = Akaikes information criteria, Log L R = log likelihood ratio, 2 v 1 = model 2 versus 1, 3 v 1 = model 3 versus 1)

Group	Log L	n	BIC	AIC	Log L R	
Males						
1	-2808.80	56	6036.75	5673.59		
2	-2895.04	14	5894.87	5818.08	2 v 1	T=172.49
3	-2880.65	17	5888.86	5795.29	3 v 1	T=143.70
Females						
1	-4143.91	70	8834.25	8427.81		
2	-4366.26	14	8841.80	8760.51	2 v 1	T=444.70
3	-4250.12	18	8640.75	8536.24	3 v 1	T=212.43

Table 5: Comparison of three model alternatives testing differences between M2 formation in males and females: (1) a separate model for males and females, (2) a joint model for males and females, same α and β (3) a joint model for males and females, same α but different β. (Log L = log likelihood, n = number of parameters, BIC = Bayesian information criteria, AIC = Akaikes information criteria, Log L R = log likelihood ratio, 2 v 1 = model 2 versus 1, 3 v 1 = model 3 versus 1)

Group	Log L	n	BIC	AIC	Log L R	
Sub-Saharan Africa						
1	-2251.96	28	4706.09	4559.92		
2	-2268.68	14	4638.45	4565.37	2 v 1	T=33.45
3	-2265.69	15	4639.68	4561.37	3 v 1	T=27.46
Malaysia						
1	-1698.83	28	3589.81	3453.65		
2	-1714.02	14	3524.11	3456.04	2 v 1	T=30.38
3	-1708.10	15	3519.14	3446.20	3 v 1	T=18.54
White UK						
1	-1583.32	28	3359.83	3222.64		
2	-1611.78	14	3320.12	6251.56	2 v 1	T=56.92
3	-1601.83	15	3307.15	3233.65	3 v 1	T=37.02
Bangladeshi UK						
1	-905.17	28	1990.14	1866.34		
2	-917.63	14	1925.15	1863.25	2 v 1	T=24.91
3	-916.57	15	1929.46	1863.14	3 v 1	T=22.80

DISCUSSION

The mandibular third molar root development was studied on a convenience collection of dental radiographs from several world regions including Sub-Saharan Africa, Japanese, Malaysian, White/ European UK and Bangladesh UK. The material included radiographs from children and adolescence aged 10-25 years and in this study seven stages of M3 root development (Ri to A½) were considered. We excluded stage apex closed, because once the root is mature, age cannot be estimated from development.

Our results show the existence of small differences between M3 root stages between ethnic groups in both males and females. However, because of the large age variation in all M3 tooth formation stages, these small differences have little impact on the 95% confidence interval of estimated age. Similarly, our results show the existence of small differences between M3 root stages between males and females in each ethnic group, with the exception of Malaysians. These difference in the timing of M3 root stages are smaller between males and females than between ethnic groups.

Strengths of this study include the world regions that are represented in the sample and the statistical approach using appropriate methods of analyses. Reference data that use descriptive statistics of mean age within a tooth stage (condition on tooth stage) are no longer considered appropriate to estimate age.[19,20,23,29] Recent developments in Bayesian methods (condition on age) and the use of a uniform age distribution avoid the problem of age mimicry and are gaining acceptance as the correct approach to estimate an age distribution, given a specific tooth stage. The 95% confidence interval for root stage of M3 can be as much as 9 years and many methods estimate age with bias.[24,27]

We aimed for a wide age range and a fairly uniform sample, but this was not always possible. The limitations of this study include insufficient number of Japanese males for analysis and no knowledge of socio-economic status or other factors. Another limitation is the subjective estimation of root fractions as well as the effect inter-observer reliability may have had on the results.

The estimated mean age in Table 2 indicate that the Sub-Saharan African males and females are slightly earlier in their M3 development than other groups. This is in agreement with previous studies that use descriptive statistics to show individuals of African origin are slightly earlier in tooth development.[5,6,8,10,11] Our study confirms these previous findings with more robust statistics. The magnitude of these differences, means that the significant difference in timing of M3 formation between ethnic groups in males has little impact on the confidence interval of estimated age.[21,22] Our findings suggest that population specific reference data for the timing of M3 in males are probably unnecessary, particularly in light of the wide 95% confidence intervals.

CONCLUSION

This study showed small differences in the timing of root development in M3 between world groups, however, because of the large standard deviations in age for each M3 root stage and therefore large 95% confidence interval, this has little impact on estimating age using M3 root formation. This suggests that a reference data set (with a wide age range and uniform age distribution) and appropriate statistical approach are probably more important than a population specific convenience sample to estimate age of an individual using M3.

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SUPPLEMENTAL METHODS

The age given stage distributions were derived in a two-step procedure. In the first step we fitted a probit model for categorical data. We let the molar stages depend upon ln(age), where ln is the natural logarithm. From this probit model analysis we obtained the mean transition ages between stages and the (common) standard age deviation for transitions. A model for the molar stages, as dependent upon age, was fitted in step two, giving the age distribution for each stage. The first step was carried out using the statistical software R and the second step was carried out using WinBUGS.

Step 1: Categorical data analysis

The purpose of the first step was to obtain the mean age for transition between stages and the (common) standard age deviation for transitions. For each molar for each ethnic group and gender we considered a data matrix containing two columns; the first contained the individual's actual age and the second the recorded molar stage (stages j=1, ..., J).

A probit model is a regression model where the dependent variable can only take a known set of discrete values, such as molar stages (in a bivariate probit model there are only two discrete outcomes, in a multivariate model there are several outcomes). The probit model can be written as

$$probit\left(stage_j\right) \sim f\left(\ln\left(age_i\right)\right) = \alpha_j + \beta \ln\left(age_i\right),$$

$$j = stage\ 1, \ldots, J-1, i = individual\ 1, \ldots, I.$$

This gave J-1 intercept estimates (α_j) and one slope parameter (β). The estimated mean age of transition between the stages j and (j+1) (on the natural logarithmic scale) are given by $\hat{\mu}_j = \hat{\alpha}_j / -\hat{\beta}$, j=1,..., J-1. The estimated common standard deviation for transitions (on the natural logarithmic scale) are given by $\hat{\sigma} = 1/-\hat{\beta}$. Hence, the estimated mean age for transition between stage one and two, on the natural logarithmic scale, is given by $\hat{\alpha}_1 / -\hat{\beta}$.

Step 2: Conditional age distribution

The purpose of this step was to obtain the age distribution for each stage. We assumed the stages to be multinomial distributed given age. p_{ij} denoted the probability for the tooth to be in stage j given age_i. The p_{ij}'s are given by

$$p_{i1} = 1 - \Phi\left(\frac{\ln\left(age_i\right) - \hat{\mu}_1}{\hat{\sigma}}\right), for\ stage\ j = 1$$

$$p_{ij} = \Phi\left(\frac{\ln\left(age_i\right) - \hat{\mu}_{j-1}}{\hat{\sigma}}\right) - \Phi\left(\frac{\ln\left(age_i\right) - \hat{\mu}_j}{\hat{\sigma}}\right), for\ j = 2, \ldots, J-1$$

$$p_{iJ} = \Phi\left(\frac{\ln\left(age_i\right) - \hat{\mu}_{J-1}}{\hat{\sigma}}\right), for\ stage\ J.$$

The estimated parameters $\hat{\mu}_1, \ldots, \hat{\mu}_{J-1}$ and $\hat{\sigma}$ were obtained in the first step, so the only unknown in these equations were the ages. The age for a given stage was uncertain, and we chose to express this uncertainty a priori by letting age_i be uniformly distributed between 0 and 110.

We fitted our model using WinBUGS by performing initial 250 000 simulations (so-called burn-ins and hence disregarded) and then another 250 000 iterations, where we retained every 100th iteration.

Fitting this model in WinBUGS gave the posterior distribution for age given stage. In this procedure the age estimates for the lowest and highest stage was bounded (the lowest by 0 and the highest by 110). What should a priori be set as boundaries for age is debatable, and a general idea is that 0 and 110 is "sufficiently large". From the model it is clear that the estimate of the lowest and highest stage will be influenced by the lower and upper bound in the prior distribution, how much of course depends upon the observed data. As a consequence, the estimated age distributions for the lowest and highest stages should be disregarded as they are clearly affected by these boundary choices (in this case stages up to crown complete and then apex closed for M3).

Comparisons between skeletal and dental age assessment in unaccompanied asylum seeking children

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KEYWORDS

age assessment, dental age, skeletal age, agreement, independent observers

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ABSTRACT

Background: For children with disputed date of birth, age assessments based on skeletal and dental development are recommended.
Aim: The aim of this retrospective study was to compare and contrast the results of age assessments from these two methods performed on unaccompanied asylum seeking children in Norway. In addition the aim of the analysis was to see if the skeletal age assessment from hand-wrist was operator sensitive.
Materials and methods: Age assessments performed from January 2010 to December 2014 were analysed. Skeletal development of hand-wrist was graded according to Greulich and Pyle (1959). Dental development of the wisdom teeth was scored on orthopantomograms according to Moorrees, Fanning and Hunt (1963) and age assessed from tables published by Liversidge (2008) and Haavikko (1970). In the statistical analysis agreement between the two age assessments was defined according to the asylum seeker's age being assessed to be older or younger than 18 years. The statistical analysis included 3333 boys and 486 girls.
Results: The agreement was 83% for boys and 79% for girls. Approximately 70% of the boys and girls were 18 years or older by both methods. It was more common that the skeletal age was assessed older than 18 years and dental age younger than 18 years for both genders. It could be demonstrated that the age assessment based on skeletal maturation was not operator sensitive.
Conclusion: The analyses demonstrate that there is good agreement between the two age assessments, but a method to combine the results would increase the reliability of the age assessments.

INTRODUCTION

An unaccompanied minor is a child without the presence of a legal guardian. In immigration law unaccompanied minors or children are generally defined as foreign nationals or stateless persons below the age of 18 years. They arrive on the territory of a state unaccompanied by a responsible adult. It also includes children who are left unaccompanied after they entered the territory of state.[1] Unaccompanied asylum seeking children (UASC) require protection, but have special rights different from adult asylum seekers. Many UASC come from countries disrupted by war and civil conflicts. The birth of the child is frequently not registered and they arrive in Europe without documentation of age. According to The Universal declaration on Human rights (1948) everyone has the right to know their age and it is the duty of the country in which they apply for asylum to try to find their chronological age by the best methods available. Some of these children appear to be older than the given age and many European countries perform an age assessment procedure.[2]

Age assessment includes some form of measurement or grading of the development from childhood to an adult fully grown person and relates the measurement to chronological age. The recommendations are that age assessments should be performed from more than one independent physical trait in the same individual.[3] There is however no standard or scientific approach for combining the results of different methods. Existing reference datasets only contain one type of measurement per person (either bone or teeth). In addition, existing tables are based on limited datasets and the description of associated uncertainties is incomplete.

Age assessments from skeletal development in children and young adults are commonly graded from the maturation and closure of the bony symphysis. The most widely used method is grading the development of the symphysis in the hand and wrist, but clavicle and ribs are also used.[4-7] The atlas by Greulich and Pyle has widespread acceptance.[4] It is based on 1000 radiographs of children and contains reference images of left hand and wrist for boys and girls separately. Along with the reference radiographs are explanations regarding the gradual age related changes observed for each image. Age from skeletal development is assessed by comparing radiographs of the non-dominant hand of the subject with the nearest matching reference radiograph provided in the atlas.

In 1955 Gleiser and Hunt graded dental development from radiographs of the dentition.[8] The grading system was further developed by Moorrees, Fanning and Hunt (MFH) and since then this grading of tooth development has been used with variations in stages from 10 to 14.[9] The principle of these grading systems is that each stage of crown or root development corresponds to a mean or median age for that stage. This applies to all 32 teeth. In adolescents and young adults all teeth except for the wisdom teeth have completed development and grading is only possible from the four wisdom teeth.

There is international agreement that the recommendation is to use two or more independent age assessment methods.[10, 11] A study from 1956[12] found a high correlation between scoring skeletal development according to Greulich and Pyle[4] and scoring dental development from first molar according to Gleiser and Hunt[8] for children aged 8 - 16 years. A Swedish study from 1971 using different tables and grading systems also showed a good agreement (r = 0.88) for children aged 6.5 -14.5 years.[13] Later studies have confirmed this agreement for children in the same age groups using various grading systems for skeletal and dental developments as well as different statistical analysis.[14-19] Few studies have compared age assessment from skeletal development and development of third molars.[7, 20-23] Gelbrich et al.[18] have demonstrated that the skeletal maturation is independent from dental development and consequently these two age markers can be combined in age assessment.

The aim of the present study has been to compare and contrast the mean or median from two biologically independent age assessments performed on UASC in Norway. The age assessment is based on analysis of hand-wrist radiographs and dental examination. In addition the aim of the analysis was to see if the skeletal age assessment from hand-wrist was operator sensitive. The study is aimed at getting a better understanding of variations and differences between the two biological methods used for age assessment in Norway.

MATERIALS AND METHODS

The material consisted of all the dental and skeletal age assessments of age disputed UASC which have been performed in Norway between January 2010 and December 2014. These UASC had age assessment performed as part of their asylum application procedure in Norway and their chronological ages were unknown. The skeletal age assessments from these examinations were based on radiographic images of hand-wrist assessed by two consultants at the Radiology Department at Oslo University Hospital from January 2010 to July 2013 and by mainly one consultant at Unilabs, Oslo from July 2013 to December 2014. The dental age assessments were performed at the Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, and carried out by four specially trained dentists and quality controlled by one dentist based on clinical and radiographic examination of the dentition. These two different biological age assessments were made on the same day by independent observers.

The radiographers assessed age from the fusion of the distal symphysis of the ulna which was graded according to the atlas by Greulich and Pyle.[4] For the purpose of this article the age assessments based on radiographs of the hand-wrist are called "skeletal age".

An oral examination was also performed and an age assessment given based on the clinical impression. From an orthopantomographic radiograph (OPG) the development of the mandibular third molars was graded according to MFH stages as described by Liversidge.[24] Age assessments were made from the development of the mandibular third molars (FDI nomenclature 38 and 48) according to the tables of Liversidge [24] and for maxillary third molars (FDI nomenclature 18 and 28) from the tables of Haavikko.[25] The dental age was given as the average age of the four assessments reduced to the nearest integer figure with the exception of one (or in few instances two) teeth being obviously delayed in development. When there was a marked discrepancy between clinical impression and assessment age from tooth development the assessed age was adjusted accordingly. For UASC with congenitally missing third molars root development on the second molar was graded and age assessed according to the tables by Haavikko, and in addition the age was calculated from the size of the pulp cavity on periapical radiographs on maxillary central incisors.[26] A collated dental age assessment was given based on clinical and radiological evidence in this article called "dental age.

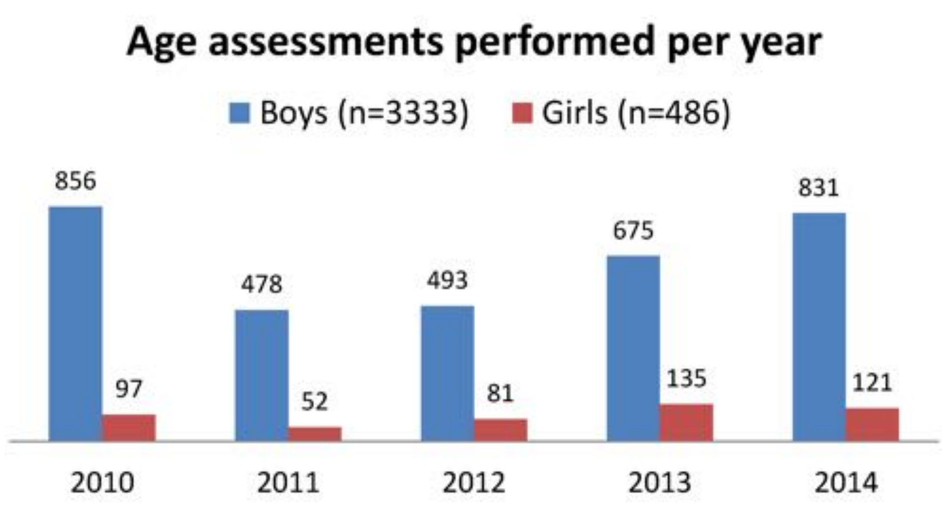
According to the atlas of Greulich and Pyle[4] the mean age for closure of the symphysis in the left hand are 18 years in girls and 19 years in boys. After this age a radiographic examination cannot tell anything more than that a girl is 18 years or older or that a boy is 19 years or older. In these cases, skeletal age is set to 18 years for girls and 19 years for boys. The age assessments were given as integers since this reflects the biological variation in development on which the age assessments were based.

Agreement between the two age assessments is defined according to the asylum seeker's age being assessed to be older or younger than 18 years. According to the UN Convention on the Rights of the Child (1989) this age differentiates a child from an adult and consequently their legal rights and obligations. There were four possible outcomes:

- *Agreement 1:* The UASC is 18 years or older from both age assessments.
- *Agreement 2:* The UASC is younger than 18 years from both age assessments.
- *Mismatch 1:* The UASC is 18 years or older from the skeletal age assessment and younger than 18 years from the dental age assessment.
- *Mismatch 2:* The UASC is younger than 18 years from the skeletal age assessment and 18 years or older from the dental age assessment.

The Mann-Whitney U test was used to compare the means of two independent groups of samples that do not necessarily follow a normal distribution. The significance level was 5%.

Figure 1: Number of age assessments per calendar year performed from January 2010 to December 2014



RESULTS

In total 3819 UASC were included in the statistical analysis, 3333 boys and 486 girls. From the first period there were 2416 UASC- of which 2124 were boys and 292 girls and from the second period 1403 UASC- of which 1209 were boys and 194 girls. Figure 1 shows the number of age assessments performed per year from 2010 to 2014 separated by gender.

Figure 2 shows the percentage of agreement and mismatch for boys and girls, respectively, for the age assessments performed from 2010 to 2014. The agreement was 83% for boys and 79% for girls and for both genders approximately 70% of the UASC were18 years or older by both methods (Agreement 1).

The mismatches showed that it was more common that the skeletal age was assessed older than 18 years and dental age younger than 18 years (Mismatch 1) for both genders. The discrepancy between skeletal age and dental age varied from -5 to 6 years and was more than one year in 11.6% of the boys and 12.6% of the girls (Figure 3).

Figure 2: Percentage of individuals with and without agreement for age assessments performed from 2010 to 2014. Agreement is defined according to the asylum seeker's age being assessed to be older or younger than 18 years. (A) Males. (B) Females

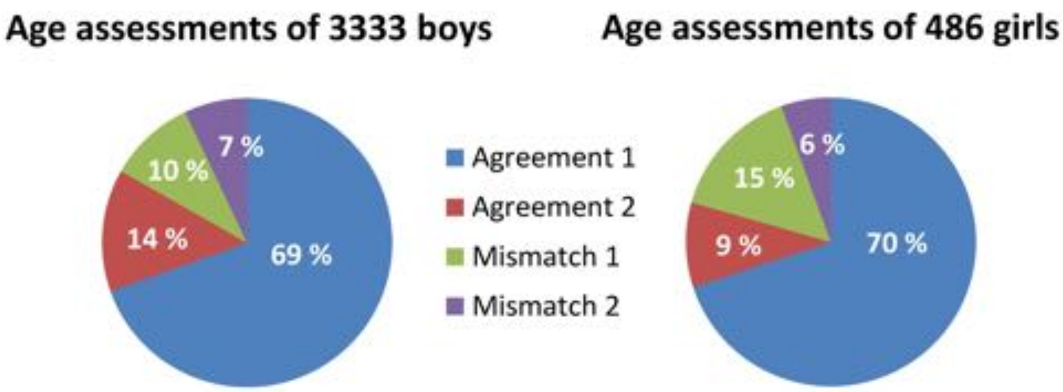
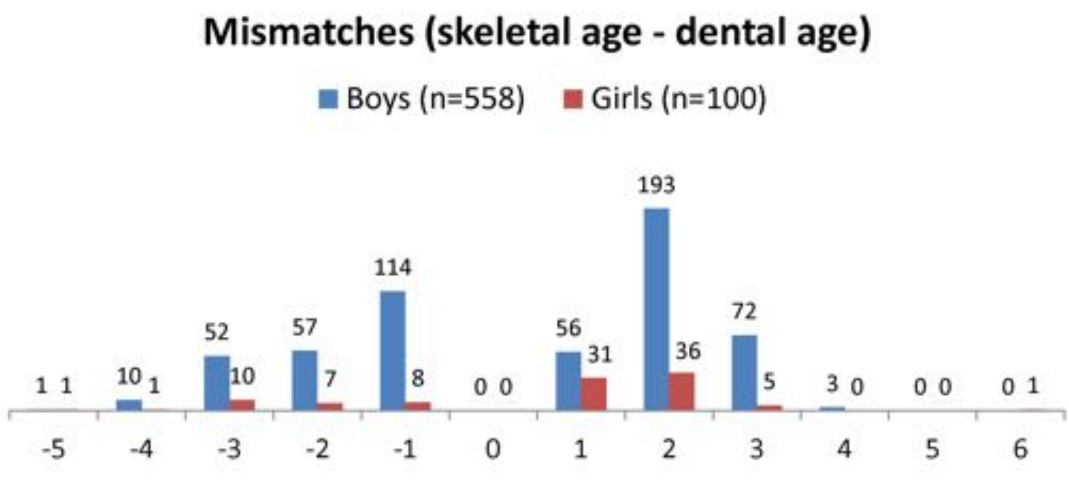


Figure 3: Mismatches between the two age assessments (skeletal age minus dental age) for both genders. For negative deviations skeletal age is lower than dental age and for positive deviations skeletal age is higher than dental age



Comparison between the two periods when radiologists from two different institutions assessed the skeletal age shows that the agreements for girls were 79.5% and 79.4%, respectively. In the same two periods the agreements for boys were 86.3% and 77.9%. The difference between the two agreements for boys is -8.4 with a 95% confidence interval from -11.2 to -5.5. The significant reduction in agreement for boys reflects that the percentage of UASC with skeletal age younger than 18 years and dental age 18 years or older (Mismatch 2) has increased from 4.0% in the first data period to 12.4% in the second data period.From the Mann-Whitney U test, the skeletal age assessment is significantly higher than the dental age assessment in both data periods for girls, so there has been no change over time in the relationship between the two age assessments for girls. The statistical tests also show that the skeletal age assessment is significantly higher than the dental age assessment in the first data period for boys. In the second period there were a larger proportion of boys with a lower skeletal age assessment than dental age assessment. Although there was no significant difference between the two age assessments in the second period, there has been a change over time in the relationship between the two age assessments for boys. There are several factors that may explain this change. Firstly, there is a significantly larger group of UASC with older age markers in the first data period compared to the second period (Figure 4). In the first data period 77.4% of the boys have a combined age assessment of 18 years or older. In the second period it is 59.2%.

Secondly, in both periods boys from Afghanistan, Somalia or Eritrea represented 81.7% and 80.4% of the total number of age assessments. However, the number of UASC- from these countries varied in the two periods, as shown in Table 1. Table 2 shows the agreement between the dental and skeletal age assessments in the two periods, for all countries of origin together and separately for Afghanistan, Somalia and Eritrea. These data provide no basis to conclude that there have been different interpretations among the operators performing the skeletal age assessments in the two data periods.

Figure 4: Combined age assessments for boys in the two data periods, separated in nine different age groups

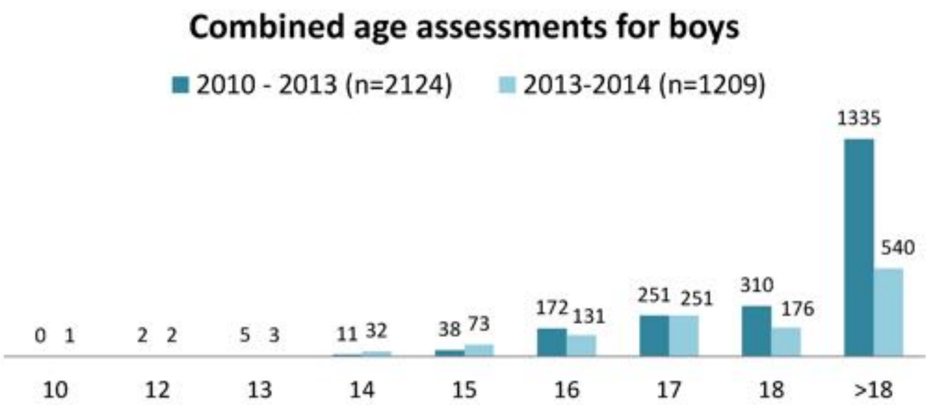


Table 1: Percentage boys from the three major countries of origin over the two periods

	Afghanistan	Somalia	Eritrea	Sum
January 2010 – July 2013	59.6%	17.7%	4.5%	81.7%
August 2013 – December 2014	29.4%	19.8%	31.2%	80.4%

Table 2: Percentage boys with agreement between dental and skeletal age assessments over the two periods, for all countries of origin together and separately for the three major countries of origin. Agreement is defined according to the asylum seeker's age being assessed to be older or younger than 18 years

	All	Afghanistan	Somalia	Eritrea
January 2010 – July 2013	86.3%	84.6%	89.9%	80.2%
August 2013 – December 2014	77.9%	76.1%	89.5%	69.0%

DISCUSSION

This retrospective study confirms that there were more boys than girls arriving as UASC in Norway. They were mainly from Afghanistan, Somalia and Eritrea which are countries where the infrastructure including registration of births are hampered by wars and conflicts and the children cannot document their age. The figures do not reflect the immigration in 2009 and 2010 as there was a considerable backlog in age assessment from previous years and for the whole period only age disputed children had an age assessment performed. This might explain the high figure of UASC with age assessment older than 18 years. In 2012 the Norwegian government introduced new regulations regarding UASC permission to remain in Norway after they turned 18 years. Only children, who were younger than 16 years when their application was granted, were guaranteed permission to remain in Norway. This might partly explain the lower average age for boys in the second data period (Figure 4).

The most common biological methods to assess age in children and young adults are to grade the dental and skeletal development. A scientific method to combine these two individual biological markers has not been devised and age assessments are either based on the average of the methods or the lowest age assessment is chosen. The chronological age in this retrospective study was unknown but the number of age assessments is considerably higher than in other studies. This allows for a comparison between the two methods. The agreement between the two methods was 83% for boys and 79% for girls. This agreement strongly supports the claim that the combined age assessment is correct. In 11.6% of the boys the difference in age assessment between the two methods was more than one year whereas this was the case for 12.6% of the girls. Although girls seem to show a greater variation in the timing of dental development,[24 25] there was little difference between the genders in this review.

Both the skeletal and dental age assessments are based on a grading system with defined stages in the development from immature to full maturation. This implies that the development is registered in a stepwise manner and the two age assessment systems are not synchronised. Therefore a one year difference might not express a real difference, but a mismatch in timing between the grading systems.

Dental age assessments in adolescents with congenitally missing wisdom teeth are difficult. The maxillary second molar is, according to Haavikko, fully formed at the age of 16.2 years for boys and 15.1 years in girls. The age assessment based on the pulp size of central incisors according to Kvaal[26] will in these age groups greatly overestimate age, but the pulp size will indicate whether the applicant is a teenager or an adult.

The atlas of Greulich and Pyle is widely used in age assessment and it has been shown that there is good agreement between operators.[27] Although skeletal development is influenced by malnutrition and starvation this deprivation has to be longstanding and catch-up to normal growth is restore once normal calorie intake is restored.[28] Teeth are less influenced by nutritional status,[29] but wisdom teeth show greater biological variation than the other teeth in a developing dentition.[25 30] Age assessments using the Greulich and Pyle Atlas or from wisdom teeth show a wide 95% dispersion.[27 30] The development of wisdom teeth shows slightly wider variations than age assessments from the Greulich and Pyle Atlas. This retrospective study demonstrates that in practical cases there is a high level of agreement between the age assessments derived from skeletal and dental development as has been shown in other studies.[19 22]

This study is not directly comparable with previously documented comparisons between dental and skeletal age markers. Most of the studies have been on younger children up to 15 years of age which have had hand-wrist and dental radiographs on the same day in preparation for orthodontic treatment,[14-18] and one study included deceased children.[19] In the present study very few UASC were assessed to be younger than 14 years. For both skeletal and dental age assessment the confidence interval increased with age and is widest for age assessments performed on third molars in the last stages of root development.[25]

Kullman et al.[21] looked at the correlations between the different age assessment methods in the 12 - 19 years age group and found better correlation with chronological age using skeletal age assessment methods rather than age assessment based on development of third molar. It is encouraging that in this retrospective study the agreement of the two methods was so high considering different factors controlling growth and development[18].

CONCLUSION

Age assessments from either Greulich and Pyle Atlas or from development of wisdom teeth demonstrate a standard deviation of approximately one year but the 95% confidence interval varies from 4.7 to 6.8 years in young adults.[27 30] These wide variations might be narrowed if the two methods could be combined. Until such tables can be produced the combinations of two independent age assessments demonstrate close agreement based on the available methods.

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Forensic age estimation based on development of third molars: a staging technique for magnetic resonance imaging

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ABSTRACT

Background: The development of third molars can be evaluated with medical imaging to estimate age in subadults. The appearance of third molars on magnetic resonance imaging (MRI) differs greatly from that on radiographs. Therefore a specific staging technique is necessary to classify third molar development on MRI and to apply it for age estimation.
Aim: To develop a specific staging technique to register third molar development on MRI and to evaluate its performance for age estimation in subadults.
Materials and methods: Using 3T MRI in three planes, all third molars were evaluated in 309 healthy Caucasian participants from 14 to 26 years old. According to the appearance of the developing third molars on MRI, descriptive criteria and schematic representations were established to define a specific staging technique. Two observers, with different levels of experience, staged all third molars independently with the developed technique. Intra- and inter-observer agreement were calculated. The data were imported in a Bayesian model for age estimation as described by Fieuws et al. (2016). This approach adequately handles correlation between age indicators and missing age indicators. It was used to calculate a point estimate and a prediction interval of the estimated age. Observed age minus predicted age was calculated, reflecting the error of the estimate.
Results: One-hundred and sixty-six third molars were agenetic. Five percent (51/1096) of upper third molars and 7% (70/1044) of lower third molars were not assessable. Kappa for inter-observer agreement ranged from 0.76 to 0.80. For intra-observer agreement kappa ranged from 0.80 to 0.89. However, two stage differences between observers or between staging sessions occurred in up to 2.2% (20/899) of assessments, probably due to a learning effect. Using the Bayesian model for age estimation, a mean absolute error of 2.0 years in females and 1.7 years in males was obtained. Root mean squared error equalled 2.38 years and 2.06 years respectively. The performance to discern minors from adults was better for males than for females, with specificities of 96% and 73% respectively.
Conclusion: Age estimations based on the proposed staging method for third molars on MRI showed comparable reproducibility and performance as the established methods based on radiographs.

INTRODUCTION

Staging third molars' development

The development of third molars can be evaluated with medical imaging to estimate age in subadults by allocating developmental stages. A staging method should cover the entire maturation sequence of the structure that is evaluated.[1, 2] Thevissen et al. (2013) pointed out that the choice of staging method should depend on the number of stages in the period of interest.[2] Moreover, stages should be defined unambiguously with clear threshold between them.[2] In forensic context, the most important question to be answered is whether or not the individual is a minor or an adult. Therefore, in most countries, an adequate staging method should encompass stages defined by changes that occur around the 18th birthday. A balance should be sought between a comprehensive method with a sufficient number of stages and a performant method with sufficient reproducibility and accuracy.[3] Stage characteristics should be straightforward and simple, in order to facilitate the learning process for observers and to exclude stage overlap caused by different interpretations.

Numerous staging techniques have been described, all of them based on radiological appearance of developing teeth.[4, 5] In all published papers on dental age estimation based on MRI of third molars, the radiological staging methods were extrapolated without any MR specific validity testing.[6-10] All of them used the Demirjian staging technique, whereas De Tobel et al. (2017) used both the Demirjian and the Köhler technique.[11, 12] Demirjian stages are defined by objective criteria, while Köhler stages are based on predictions of crown and root lengths. De Tobel et al. reported considerations to take into account when transferring Demirjian and Köhler stages to MRI. Still, major concerns remain regarding the different appearance on MRI compared with radiographs, which cannot be overcome using the existing staging techniques. Therefore, a specific staging technique is necessary to classify third molar development on MRI and to apply it for age estimation.

Statistical approach to age estimation

It has been stated that a Bayesian approach renders the most appropriate age estimation using developmental stages.[13, 14] Although the prediction outcome does not strongly outperform the classical regression result, it circumvents some assumptions that are not true in age estimation: (1) a linear relationship between age and stages, (2) a normal distribution of the variation of age around the mean with a constant variance and (3) uncorrelated development of the different anatomical structures[13, 15]. The major drawback of a Bayesian approach is its computational burden when combining multiple dependent predictors. However, this can be circumvented. Fieuws et al. (2016) reported a practical approach using Bayes' rule combining multiple age indicators based on Boldsen et al. (2002).[16, 17] The ad-hoc procedure allows to construct an approximate confidence interval without the need to model the multivariate correlation structure between the indicators.[16]

Aims

The aims of the current study were (1) to develop an MRI specific staging technique for the development of third molars and (2) to evaluate the age estimation performance of a Bayesian approach using this MRI specific staging.

MATERIALS AND METHODS

Study population

The local ethics committee approved the study and written informed consent was obtained from every participant. In case the participant was a minor, the parents' consent was also obtained. A study sample of 309 healthy Belgian and Dutch Caucasian volunteers (163 females, 146 males) were prospectively included. Table 1 shows the age distribution of the participants per sex. Additionally, four younger children were scanned (two girls of age 7 and 11; two boys of age 9 and 13). Their images were used to illustrate certain stages, but they were not included for analyses. Part of the study population was included in previous papers.[9, 10] None of the participants were relatives up to the third degree. Neither had any of them had any removal of a third molar. Socio-economic background was documented. Teeth were named according to the International Standards Organisation Designation System.

Table 1: Number of participants per age per sex.

Age (y)	Frequency		
	Female	Male	Total
14	11	11	22
15	11	10	21
16	10	10	20
17	11	10	21
18	13	10	23
19	15	14	29
20	20	10	30
21	14	11	25
22	12	12	24
23	12	10	22
24	11	11	22
25	13	12	25
26	10	15	25
Total	**163**	**146**	**309**

Image acquisition

Between March 2012 and May 2017, 3T MRI was conducted according to the protocol described in De Tobel et al. (2017) with a Siemens scanner (Magnetom Trio Tim, Siemens, Erlangen, Germany).[9] Fast spin echo (FSE) T2 images were available in three planes. Sagittal images were made along the long axis of the teeth per side. Axial images were made parallel to the occlusal plane, whereas coronal images were made perpendicular to the occlusal plane (Figure 1). A bilateral flexible four-channel surface head coil (Model NMP-001D-ST-4, Nova Medical Inc., Wilmington, NC, USA) and an individualised bite bar were used. In ten cases (3.2%), the scan had to be done over because of motion artefacts (9 cases) or wrong coil positioning (1 case). In three cases the head positioning was too extended at the neck, causing motion artefacts because the participant could not keep the lower teeth still in the bite plate. This was resolved in the second scan by making a new bite plate, allowing for a more neutral neck position.

Image analysis

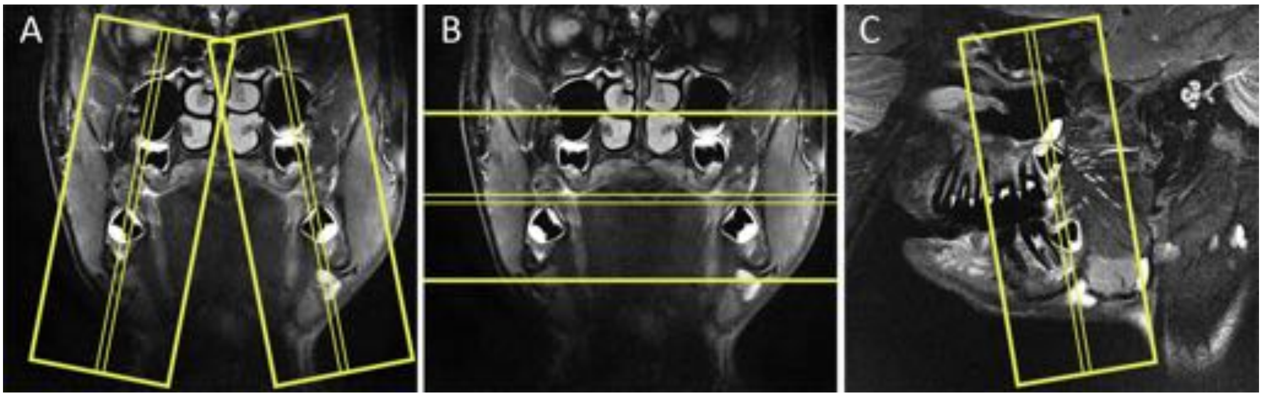
MRI specific staging technique

The considerations recommended by De Tobel et al. (2017)[10], together with other concerns made by the authors of the current study, were included to develop an MRI specific staging technique for third molar development.

The allocation of stages based on MRI should be conducted scrolling through the whole stack of slices depicting the considered tooth. When a fluid containing structure is seen in the jaw where the third molar is expected, stage 0 can be allocated. In fact, one can only be sure that a third molar is present when calcification appears (stage 1). After all, the crypt may be a cyst in which no tooth will develop (this situation is similar when evaluating radiographs). Therefore stage 0 should not be included in any analysis for age estimation. Since the youngest participants in the study sample were 14 years of age, it could be decided that the third molar was agenetic when no possible crypt or calcified tooth part was seen at the third molar region.[1, 18]

Since on regular MR-images no distinction can be made between enamel, dentin and cementum, criteria based on these materials were omitted. For instance, the cemento-enamel junction cannot be identified on MR-images. As a consequence, the MR crown height was defined as being the distance between the tips of the cusps and the pulp horns (Figures 2 and 3). Corresponding to Demirjian's rules, when the different cusps are not at the same level, the midpoint between them is considered the highest reference point. Similarly, the lowest reference point is the midpoint between the pulp horns. Lines to define MR crown height should be perpendicular to the tooth axis. New criteria based on this MR crown height were formulated.

Figure 1: Yellow boxes depict the stack of scanned MRI slices



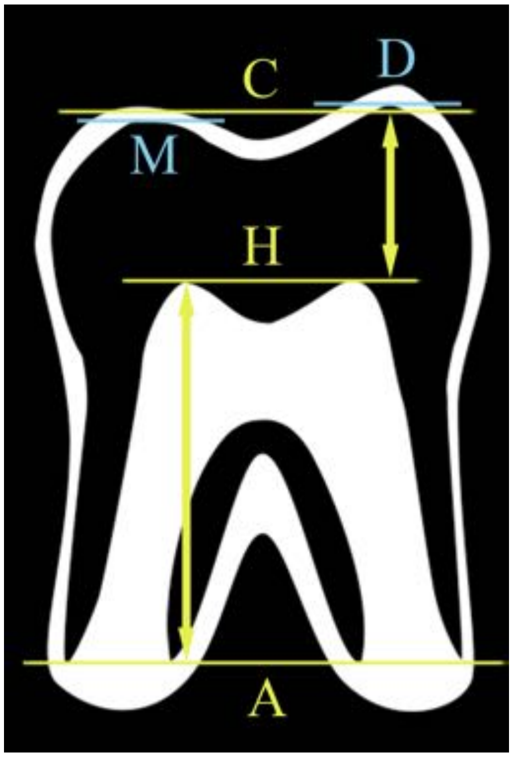


Figure 2: Definition of MR crown height and MR root length on MRI. Lines are perpendicular to the tooth axis. Distances (arrows) are evaluated along the tooth axis.
Line D is at the distal cusp tip, while M is at the mesial cusp tip. Line C represents the midpoint between the distal and mesial cusp tips.
Line H is at the pulp horns, which are both at the same level.
Line A is at the most apical point of the roots, which are all at the same line.
The distance between lines C and H is the MR crown height. The distance between lines H and A is the MR root length. In this case MR root length is more than one and a half MR crown height, so the tooth is in stage 5

The MR crown height clearly differs from the clinical crown height, which – in contrast to radiographs or CT – cannot be determined on MRI. When evaluating relative lengths, a pair of dividers can be used to compare MR root lengths with MR crown height. In borderline cases, the measure tool of the viewing software can be used to compare absolute measures. MR root length is measured from the nearest pulp horn to the most apical point of the root, at the least developed root wall (Figures 2 and 3). The least developed root should be considered in case different roots are in different developmental stages.

Stages 3, 4 and 5 depend on MR root length compared with MR crown height. In case doubt prevails even after using the measure tool, the youngest stage should be allocated. Because the measure tool can only be used on one slice, it is impossible to measure tooth proportions when the tooth is depicted over multiple images. Still, when a certain MR root length is nearly reached on one slice and

Figure 3: (A, B) Lower right mandibular third molar depicted on two consecutive MRI slices. Slice (A) is situated more buccally than slice (B). The pulp chamber has a trapezoidal shape, corresponding to stage 3. To exclude stage 4, MR crown height and MR root length have to be evaluated as illustrated in images (C, D).
(C) Copy of image (A), with marked landmarks and distances to consider in order to allocate a stage. Lines are perpendicular to the tooth axis. Distances (arrows) are evaluated along the tooth axis. Line Dc is at the distal cusp tip, while Mc is at the mesial cusp tip. Line C represents the midpoint between the distal and mesial cusp tips. Line Dh is at the distal pulp horn while line Mh is at the mesial pulp horn. Line H represents the midpoint between the distal and mesial pulp horns. Line Da is at the most apical point of the distal root. Line Ma is at the most apical point of the mesial root.
The distance between lines C and H is the MR crown height based on this slice. The distance between lines Dh and Da is the distal MR root length, while the distance between lines Mh and Ma is the mesial MR root length.
(D) Copy of image (B). Both cusp tips are at the same level on this slice, represented by line C. MR crown height is larger than on the previous image, whereas the distal MR root length is smaller.
To allocate a stage, MR crown height on image (D) is the most appropriate, while the distal MR root length on image (C) is the most appropriate. Because the third molar is tilted bucco-lingually, the observer has to scroll through consecutive slices to decide on the most appropriate measures to consider. In slice (C) the crown is transsected more buccally, so part of the crown is not depicted. By contrast, the distal root apex is situated more buccally than the crown, so it is best depicted in slice (D). Because the distal root is shorter than MR crown height, this third molar is in stage 3.

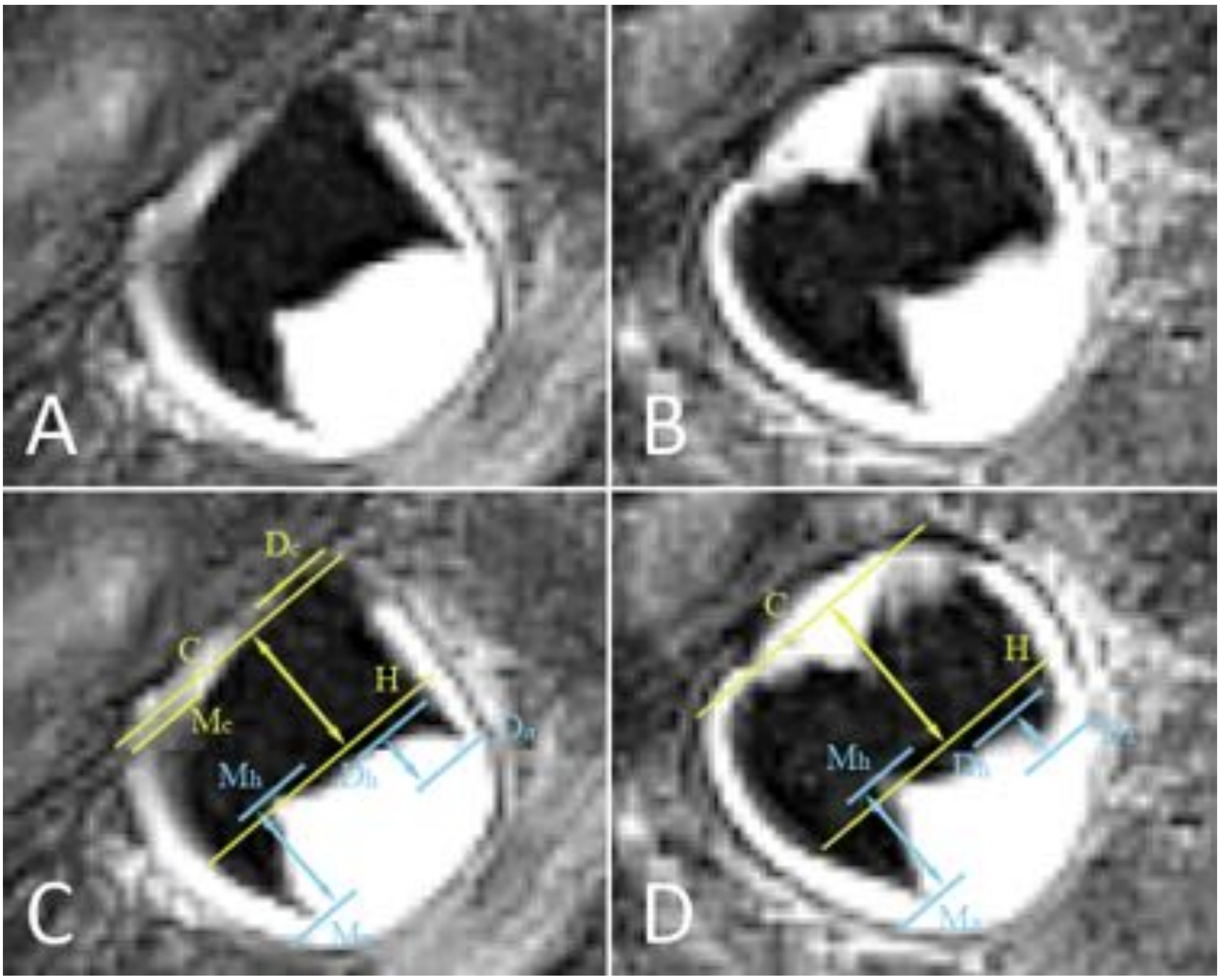
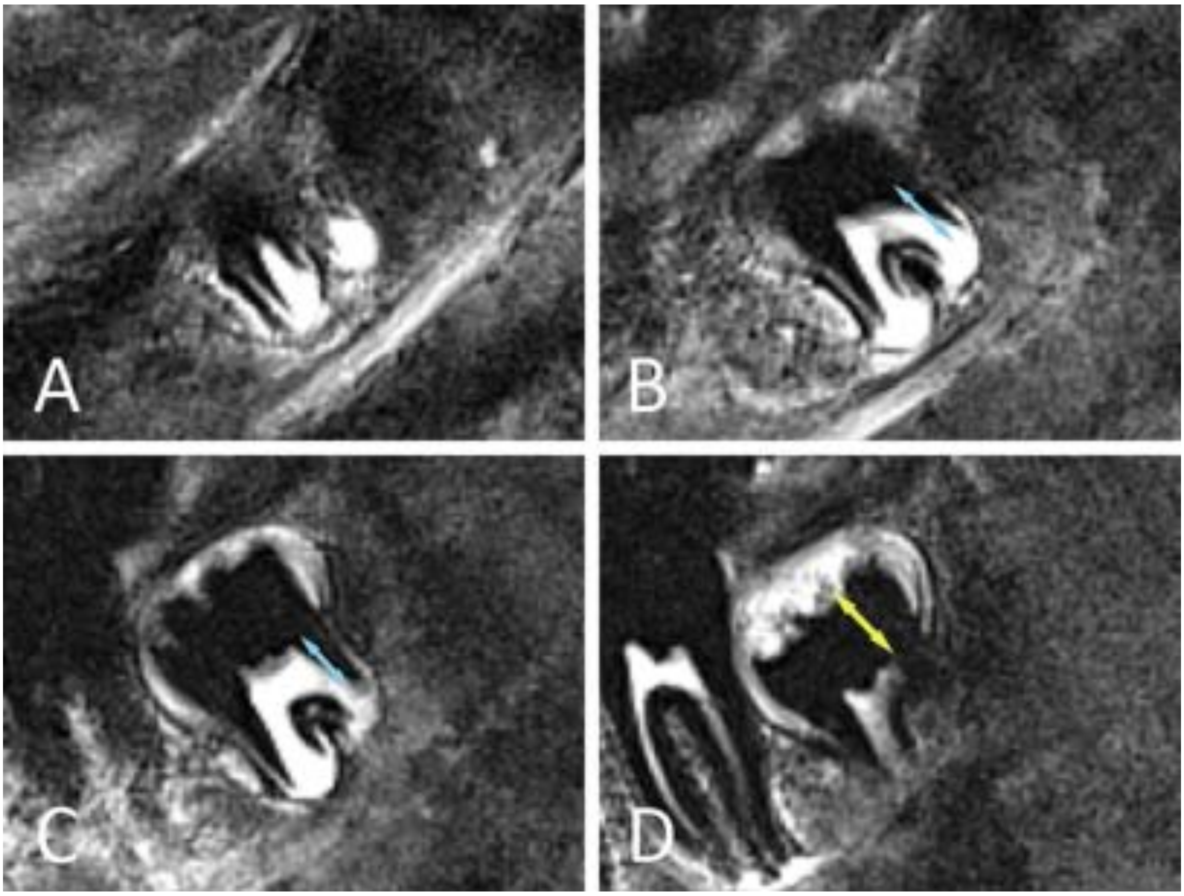


Figure 4: Lower right mandibular third molar depicted on consecutive MRI slices from buccally to lingually. MR crown height is most appropriately measured on slice D. When MR root length would only be based on slice C, stage 3 would be allocated. In fact the tooth is in stage 4, since MR root length on slice B is slightly longer than MR crown height and the root is depicted over several slices (keeping in mind that slice thickness is 2 mm).



the root is spread over multiple consecutive sagittal slices, the higher stage can be allocated (Figure 4). After all, one has to take into account that the MR-sequence has a slice thickness of 2 mm. In the final stages, the root dentin at the apex changes from thin and parallel (stage 6), over thicker and converging (stage 7), to thick and closed (stage 8).All of these considerations resulted in the stages defined in Table 2 and Figure 5. To have reached a certain stage, the appearance of the root has to comply with the given criteria. When two criteria are given, the molar has reached the stage if the first criterion applies. When three criteria are given, the first two have to apply to allocate the concerning stage. Both schematic representations of uniradicular third molars and multiradicular third molars are given. Examples of the appearance of the different stages on MRI are shown in Figures 6 to 8.

Observers and media

Images were anonymised and evaluated by two observers independently. Observers were blinded to the age of the participant. Five participants per age per gender between 14 and 26 years old were assessed in a first session. After four months, both observers evaluated all 309 participants in a second session. The first observer (J.D.T.) was a resident at maxillofacial surgery studying forensic dentistry. He had been involved in research on age estimation for 8 years, including 6 years of dental age estimation. The second observer (I.P.) was a dentist in the first year after graduation. She had been involved in dental age estimation research for 1 year.

Images were assessed using a Barco MFGD monitor (3280 x 2048 pixels, Barco, Kortrijk, Belgium).

Table 2: Descriptive criteria for developmental stages of third molars on MRI.

Stage	Description
Stage 0	The crypt of the third molar is suspected without any calcification.
Stage 1	A beginning of calcification is seen at the superior level of the crypt in the form of an inverted cone or cones. There is no fusion of these calcified points.
Stage 2	a) Fusion of the calcified points forms one or several cusps which unite to give a regularly outlined occlusal surface. b) The outline of the pulp chamber has a flat or curved shape at the occlusal border. c) Initial formation of the radicular bifurcation is seen in the form of a hypo-intense calcified point.
Stage 3	a) The pulp chamber has a trapezoidal shape. The outline of the pulp horns is pointy and shaped like an umbrella top. b) Further downshaping of the crown and/or beginning of root formation is seen in the form of a spicule. The spicule is shorter than MR crown height. c) The calcified region of the bifurcation has developed further into a hypo-intense semi-lunar shape.
Stage 4	a) MR root length reaches at least one MR crown height. b) The calcified region of the bifurcation still has a semi-lunar shape or has developed further down.
Stage 5	a) MR root length reaches at least one and a half MR crown height. b) The calcified region of the bifurcation has developed further down from its semi-lunar shape to give the roots a more definite and distinct outline with funnel shaped endings. The funnel shape persists for some millimetres (i.e. it is not limited to a few pixels on the image).
Stage 6	a) The walls of the distal root canal are parallel and its apical end is still partially open. b) The walls at the apex of the root canal show relatively thin dentin. c) Remnants of the dental follicle are seen in the form of a hyper-intense area surrounding the apex.
Stage 7	a) The walls of the distal root canal are convergent and its apical end is still partially open. b) The walls at the apex of the root canal show relatively thin dentin. c) Remnants of the dental follicle are seen in the form of a hyper-intense area surrounding the apex.
Stage 8	a) The apical end of the distal root canal is completely closed. b) The walls at the apex of the root canal show relatively thick dentin.

Figure 5: Schematic representation of developmental stages of third molars on MRI. Mineralized tissues appear black on MRI. By contrast, the dental follicle, pulpal tissues, the periodontal space and saliva appear white. The upper panels show stages for multiradicular molars, while in the lower panels stages for monoradicular molars are shown (this also corresponds with the appearance of the palatal root in upper molars).

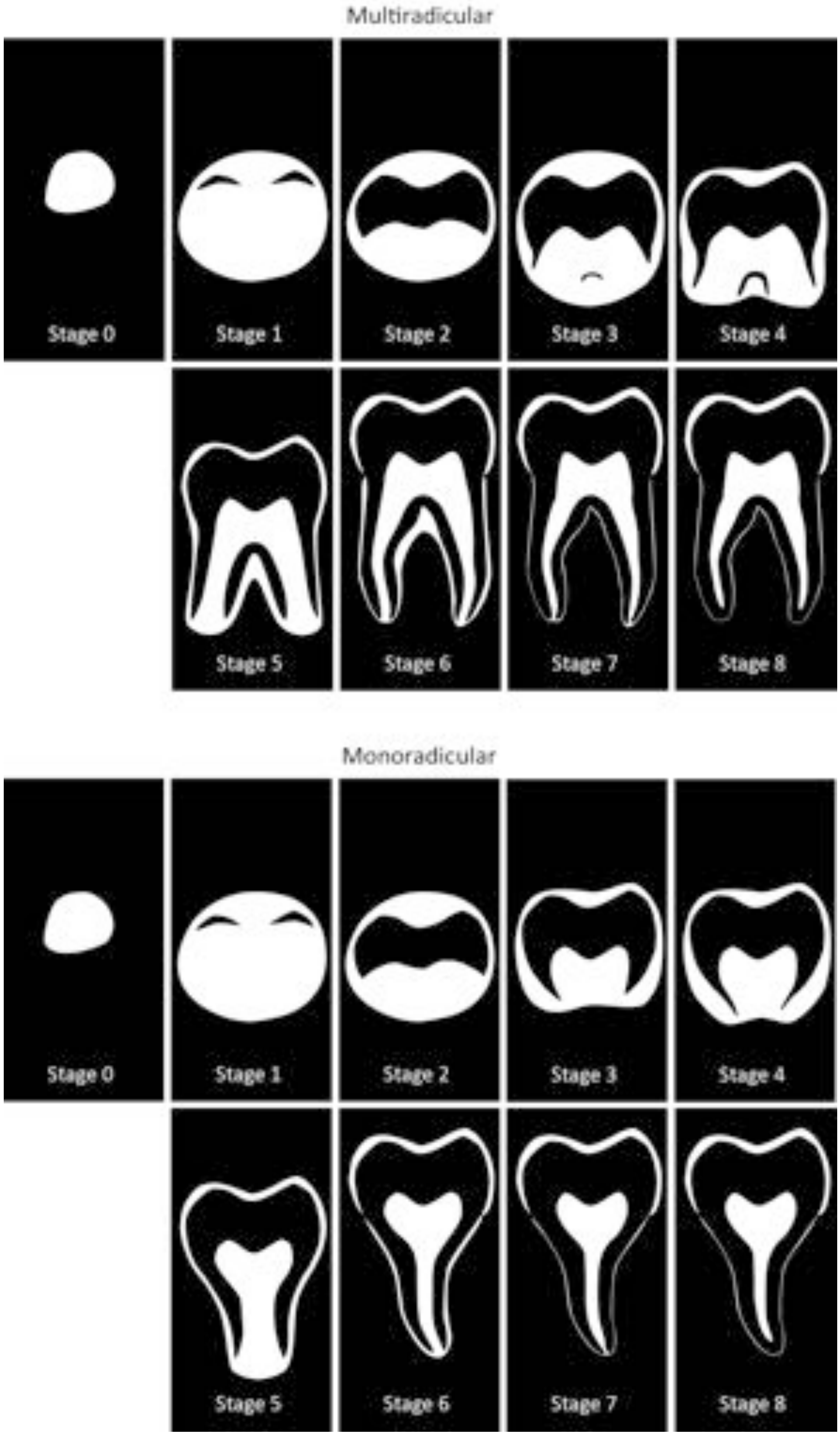


Figure 6: Representative examples of third molars in developmental stages 0 to 3, in the upper (A-E) and lower jaw (F-J). For some stages different appearances are illustrated.
(A) Stage 0. The crypt of the third molar shows no calcification. It is seen as a clearly delineated white area.
(F) Stage 1. Cusp tips are seen as separate black areas within the crypt.
(B, G) Early stage 2. Cusps are fused. The roof of the pulp chamber is quite flat.
(C, H) Late stage 2. The roof of the pulp chamber is more curved than in (B, G). Notice that in (H), the mesial side of the pulp chamber is more mature than the distal side. Thus, for staging the distal side should be considered. The distinction between early and late stage 2 was considered too subjective to consider them as separate stages.
(D, I) Stage 3. Notice the pointy appearance of the pulp horns. No furcation was present.
(E, J) Stage 3. Notice the furcation. In (J) the distal pulp horn appears curved on this sagittal slice. However, scrolling through the slices and including the coronal slices in the assessment, it was clear that both pulp horns were pointy, like an umbrella top.

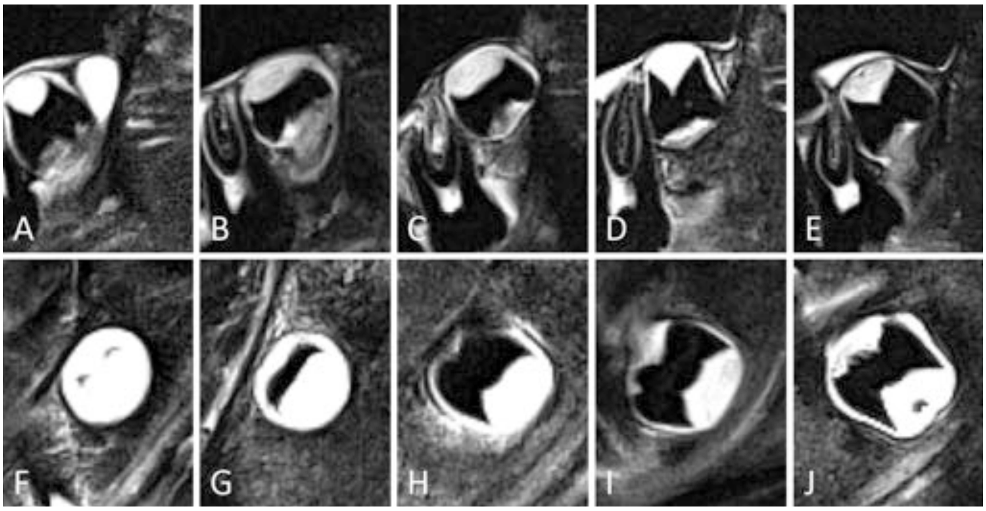


Figure 7: Representative examples of third molars in developmental stages 4 and 5, in the upper (A-C) and lower jaw (D-F). In (A-C) palatal roots are depicted. For stage 5 different appearances are illustrated.
(A, D) Stage 4. Notice that the distal root in (D) is less developed than the mesial root.
(B, E) Early stage 5. Root walls are clearly funnel shaped at the root apex.
(C, F) Late stage 5. The funnel shape of the root walls at the apex is more subtle than in (B, E). The distinction between early and late stage 5 was considered too subjective to consider them as separate stages. Moreover, variability in root length would hinder a subclassification of stage 5.

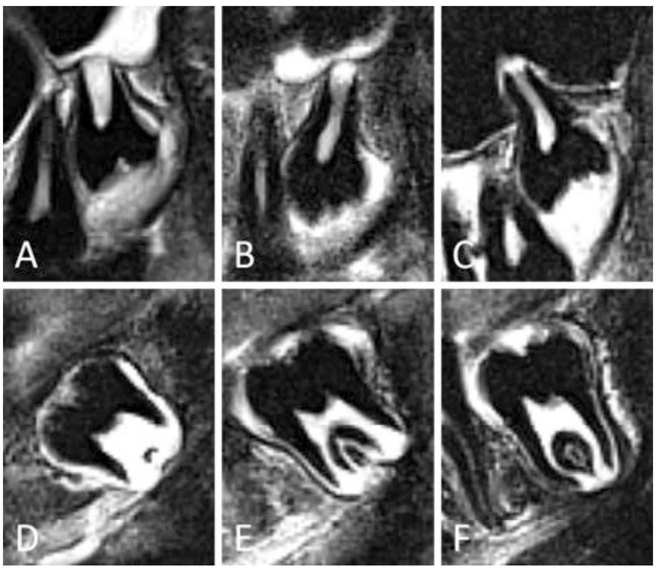
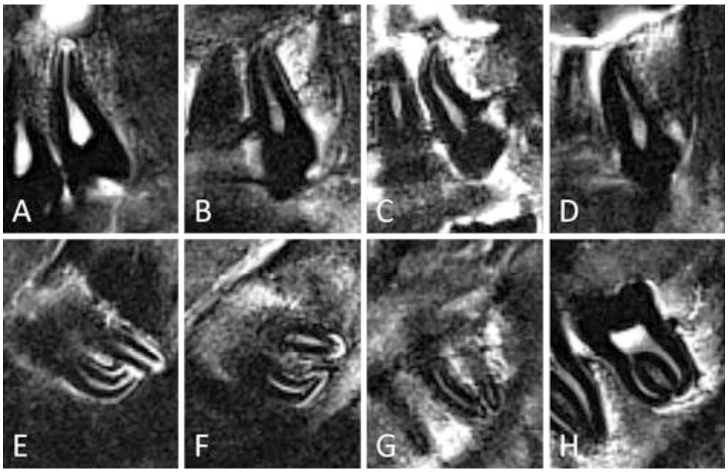


Figure 8: Representative examples of third molars in developmental stages 6 to 8, in the upper (A-D) and lower jaw (E-H). In (A-D) palatal roots are depicted. For stage 8 different appearances are illustrated.
(A, E) Stage 6. The width of the root canal differs depending on tooth anatomy. Still, parallel root walls are clear. Notice that the thin dentin at the apex in (A) might give the impression of a small funnel shape. However, it is stated in the criteria for stage 5 that the funnel shape should be more extensive than is seen in this example. Therefore, stage 6 is appropriate.
(B, F) Stage 7. The apices have clearly started closing. In (F) remnants of the dental follicle can be seen as white areas surrounding root apices.
(C, G) Stage 8. The apical dentin is relatively thin, but clearly continuous.
(D, H) Stage 8. Not only is the apical dentin continuous, but in these examples it is also relatively



Studied variables
Using Microsoft Access forms, both observers assessed the images gathering data on four variables. First, a developmental stage was allocated or it was decided that the tooth could not be evaluated. Reasons for the latter were included in Table 3.[10]
Second, it was documented which root was considered to decide on the stage. Third, assessability of the roots was noted (Table 4). Fourth, observers indicated which planes they used to allocate a stage, allowing for combinations to be ticked off.

Statistical analysis
All data were transferred from Microsoft Access to SPSS Statistics 24.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA) and SAS 9.4 (SAS Institute, Cary NC, USA). Descriptive statistics were calculated. Results of the second session by both observers were combined to report on the root used to stage, assessability of the roots and essential planes.
A paired Wilcoxon test was used to compare development between upper and lower third molars on the same side and to compare left and right third molars in the same jaw. Inter- and intra-observer agreement regarding stage allocation were quantified using proportion agreement and weighted kappa statistics. Cross tabulation of the observations allowed to check for systematic differences. The difference in marginal score distribution (between two observers or between two measurement occasions) was verified with Bowker's test of symmetry.
The data from the first observer were implemented into an ad-hoc procedure to obtain a point estimate of age and appropriate prediction intervals.[16] Participants with all available third molars in stage 8 were not included in the analysis (N = 28). Hence, the prediction pertains to subjects with not all available third molars fully developed. Motivation for this approach was that the point prediction (and thus the error) for participants with fully developed third molars is heavily influenced by the age range of included participants.
The ad-hoc procedure was based on application of Bayes' rule, using continuation-ratio models assuming conditional independence. The model takes third molar position into account, so that

Table 3: Reasons for the MRI being not assessable, with their frequencies.

Reason for being not assessable	Upper third molars		Lower third molars	
Insufficient contrast between apex tip and surrounding bone	2.6%	(28/1096)	2.1%	(22/1044)
Apex tip falls in between slices	0.5%	(6/1096)	1.8%	(19/1044)
Poor coil positioning	0.0%	(0/1096)	0.0%	(0/1044)
Poor image quality (e.g. poor signal to noise ratio)	0.3%	(3/1096)	0.4%	(4/1044)
Artefacts due to motion of the participant	0.9%	(10/1096)	1.5%	(16/1044)
Other artefacts (e.g. susceptibility due to metal)	0.4%	(4/1096)	0.6%	(6/1044)
Other, please specify	0.0%	(0/1096)	0.3%	(3/1044)

Table 4: Relative assessability of the different roots

Elements	Assessablity	Frequency	
Upper third molars	Only the staged root is assessable	4%	(32/914)
	Other roots are also assessable	81%	(739/914)
	Only the staged root is present	16%	(143/914)
Lower third molars	Only the staged root is assessable	2%	(16/844)
	Other roots are also assessable	97%	(817/844)
	Only the staged root is present	3%	(27/844)

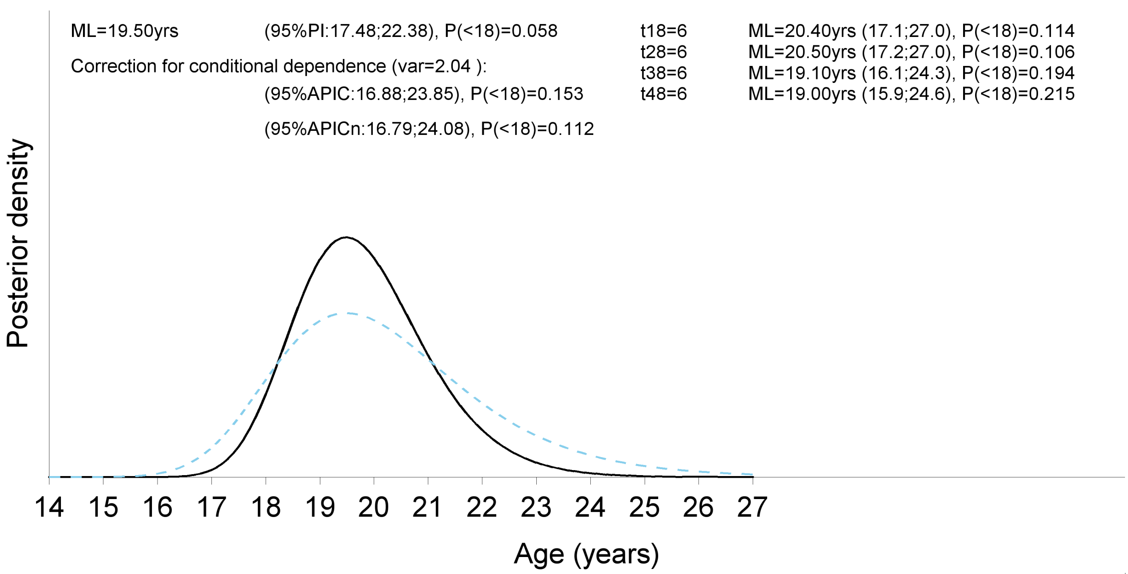
even when one or more third molars are agenetic, the other third molars contribute to the model. The influence of agenetic third molar(s) to the posterior density distribution was illustrated in Figure 9. Non-proportional odds were allowed in the continuation-ratio model. Linearity was assumed for the relation between age and the logits. Note that for the same reason, the more simplistic model assuming proportional odds would lead to a more stable solution. Due to the low number of scores equal to 2, these were combined with scores 3 into a single level. Evaluation of the performance was based on 10-fold cross-validation and the approach was performed separately for males and females. The creation of the folds was stratified on age category (1 year interval).

Observed age minus predicted age was calculated, reflecting the error of the estimate. The maximum likelihood (ML) estimate was given as point prediction (this equals the modus of the posterior distribution of age), as well as the mean and 95% trimmed mean of the posterior distribution. The interval estimate (prediction interval) for age corresponded to the 95% age values of highest probability density. The difference between the posterior density assuming conditional independence and the correction by the ad-hoc procedure was illustrated in Figure 10.The proportion of cases, whose chronological age fell inside the 95%

confidence interval (CI), known as coverage, was calculated. Since the ML estimate is known not to minimize the root mean squared error (RMSE), the mean of the posterior distribution, as well as the mean of the posterior distribution in the prediction interval (= trimmed mean) were also reported.Spearman correlation between chronological age and staging was calculated, as well as Pearson correlation between chronological age and error of the estimated age. The first reflecting the degree of change in development explained by a change in age. The latter reflecting the degree of bias of the age estimate. Accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were used to evaluate the minor-adult distinction. Accuracy represented the proportion of correctly classified subjects. Sensitivity indicated the proportion of correctly classified adults, while specificity indicated the proportion of correctly classified minors. PPV equalled the proportion of adults within estimated adults. NPV was the proportion of minors within estimated minors. The area under the receiver operator characteristic (ROC) curve (AUC) reflected the percentage of times that a randomly selected individual from the older age category would have a more advanced root compared to a randomly chosen individual from the younger age category. Finally, the AUC probability to be older than 18 years was calculated.
Statistical tests were performed two-sided and evaluated at the 0.05 significance level.

Figure 9: Influence of agenetic third molars on the posterior density. The posterior density becomes smaller as a function of increasing information (plot obtained from males).

Figure 10: Influence of agenetic third molars on the posterior density. The posterior density becomes smaller as a function of increasing information (plot obtained from males).



RESULTS

Tooth development and assessability

In the study sample, agenesis of one or more third molars was frequently seen: teeth 18, 28, 38 and 48 were agenetic in 40, 30, 50 and 46 out of 309 cases respectively (Table 5).

Tables 4 and 6 summarise results on the relative assessability of the different roots and root used to stage per third molar. The root used to stage was always the least developed assessable root.

These results included all assessable third molars from stage 4 on. No stage could be allocated in 5% (51/1096) of upper and 7% (70/1044) of lower third molars (Table 3). In a few cases monoradicular third molars were encountered (Table 4). Nineteen percent of the upper third molars had unassessable (mostly buccal) roots, compared to only 3% of lower third molars (mesial or distal were approximately equally distributed).

Table 5: Patterns of agenesis of one or more third molars with their frequencies.

Agenetic elements	Frequency
18	6
28	3
38	7
48	6
18, 28	7
38, 48	14
18, 38	2
18, 48	2
28, 38	1
18, 28, 38	1
18, 38, 48	3
28, 38, 48	2
18, 28, 38, 48	10
Total	**64**

Table 6: Frequency of root used to stage (least developed).

Elements	Root used to stage	Frequency
Upper third molars	Palatal	88% (741/838)
	Mesiobuccal	9% (75/838)
	Distobuccal	3% (22/838)
Lower third molars	Mesial	11% (91/845)
	Distal	89% (754/845)

Essential plane

Sagittal images were essential to allocate a stage in 95% (1040/1096) of assessable upper third molars. Only in a few upper third molars did the coronal (5% = 55/1096) plane contribute to staging, while the axial images were never useful. In lower third molars frequencies were 92% (966/1044), 4% (41/1044) and 1% (9/1044) for sagittal, coronal and axial images respectively. Coronal and/or axial images proved to be useful when the tooth was extremely tilted, when the apex seemed to fall in between sagittal slices or to differentiate stage 2 from stage 3.

Staging and age estimation

In fourteen participants all third molars were not allocated a stage, because they were either agenetic (n = 10) or unassessable due to motion artefacts (n = 2) or susceptibility to metal (n = 2). A systematic difference in development between upper and lower third molars was statistically confirmed ($P = 0.001$ right, $P < 0.001$ left), with lower third molars overall being in the same or more advanced stages than upper ones. Left and right third molars in the same jaw did not differ significantly in development ($P = 0.283$ upper, $P = 0.085$ lower).

Reproducibility of staging

Table 7 shows inter- and intra-observer agreement for stage allocation. Table 8 shows the cross tabulation of frequencies of allocated stages by both observers at both staging sessions. A one stage difference was frequently seen. Two stage differences were also encountered between staging sessions in 1.3% (5/379) and 1.4% (5/350) of assessments, and between observers in 1.4% (5/364) and 2.2% (20/899) of assessments. Moreover, a systematic difference in allocated stages was noticed for both observers. In the second session more frequently a higher stage was allocated. Bowker's test of symmetry indicated no statistically significant asymmetry between both staging sessions for observer 1 ($P = 0.21$), while it was significant for observer 2 ($P < 0.001$). This also resulted in a significant asymmetry between both observers in the second session ($P < 0.001$), while in the first session asymmetry was not significant ($P = 0.51$).

Table 7: Reproducibility of staging third molar development. The proportion agreement as well as two versions of the weighted kappa are reported for all third molars combined, as well for each third molar separately. The kappa with the linear weights is the weighted kappa, typically reported in most agreement studies. The kappa with quadratic weights is similar to the intra-class correlation (ICC). Note that results based aggregated data from the four third molars do not take into account the correlation between the four third molars.

Element		**Intra-observer agreement**				**Inter-observer agreement**		
		Agreement (SE)	**Weighted kappa**			**Agreement (SE)**	**Weighted kappa**	
			Linear (SE)	**Quadratic (SE)**			**Linear (SE)**	**Quadratic (SE)**
	Observer 1				Session 1			
All third molars	(N = 379)	0.760 (0.022)	0.873 (0.013)	0.954 (0.006)	(N = 364)	0.808 (0.021)	0.893 (0.012)	0.959 (0.006)
18		0.722 (0.046)	0.857 (0.025)	0.953 (0.009)		0.868 (0.036)	0.932 (0.019)	0.977 (0.007)
28		0.760 (0.043)	0.848 (0.030)	0.933 (0.017)		0.781 (0.042)	0.866 (0.028)	0.944 (0.015)
38		0.769 (0.044)	0.890 (0.023)	0.966 (0.008)		0.773 (0.045)	0.868 (0.029)	0.946 (0.015)
48		0.791 (0.043)	0.889 (0.025)	0.959 (0.012)		0.809 (0.042)	0.903 (0.022)	0.968 (0.008)
	Observer 2				Session 2			
All third molars	(N = 350)	0.760 (0.022)	0.834 (0.015)	0.954 (0.006)	(N = 899)	0.620 (0.016)	0.790 (0.010)	0.922 (0.005)
18		0.713 (0.049)	0.846 (0.028)	0.945 (0.012)		0.648 (0.032)	0.803 (0.021)	0.926 (0.010)
28		0.674 (0.049)	0.804 (0.032)	0.919 (0.017)		0.617 (0.032)	0.784 (0.021)	0.920 (0.010)
38		0.774 (0.046)	0.877 (0.027)	0.958 (0.010)		0.588 (0.034)	0.764 (0.023)	0.907 (0.012)
48		0.655 (0.051)	0.809 (0.030)	0.931 (0.013)		0.624 (0.033)	0.804 (0.019)	0.933 (0.008)

Table 8: Cross tabulation of frequencies of allocated scores by both observers in both staging sessions.

Intra-observer agreement

Observer 1

	Frequency	Stage session 1: 2	3	4	5	6	7	8	Total
Stage session 2	**2**	4	0	0	0	0	0	0	4
	3	0	52	3	0	0	0	0	55
	4	0	6	58	6	0	0	0	70
	5	0	0	11	50	1	1	0	63
	6	0	0	1	13	37	9	0	60
	7	0	0	0	1	11	30	5	47
	8	0	0	0	0	2	21	57	80
	Total	4	58	73	70	51	61	62	379

Observer 2

	Frequency	Stage session 1: 2	3	4	5	6	7	8	Total
Stage session 2	**2**	4	2	0	0	0	0	0	6
	3	1	40	1	0	0	0	0	42
	4	0	14	33	0	0	0	0	47
	5	0	0	22	76	0	0	0	98
	6	0	0	2	15	27	5	1	50
	7	0	0	0	0	6	33	11	50
	8	0	0	0	0	2	22	33	57
	Total	5	56	58	91	35	60	45	350

Inter-observer agreement

Session 1

	Frequency	Stage observer 2: 2	3	4	5	6	7	8	Total
Stage observer 1	**2**	4	0	0	0	0	0	0	4
	3	1	51	6	0	0	0	0	58
	4	0	5	47	21	0	0	0	73
	5	0	0	5	64	0	1	0	70
	6	0	0	0	5	35	4	1	45
	7	0	0	0	1	5	48	3	57
	8	0	0	0	0	2	10	45	57
	Total	5	56	58	91	42	63	49	364

Session 2

	Frequency	Stage observer 2: 2	3	4	5	6	7	8	Total
Stage observer 1	**2**	20	7	0	0	0	0	0	27
	3	5	77	30	1	0	0	0	113
	4	0	4	64	74	3	0	0	145
	5	0	0	2	112	29	0	0	143
	6	0	0	1	33	102	30	3	169
	7	0	0	0	0	33	75	14	122
	8	0	0	0	0	12	61	107	180
	Total	25	88	97	220	179	166	124	899

Age estimation

An overview of chronological age and estimated age for the study sample is presented in Figure 11. Figure 12 presents posterior distributions of the Bayesian approach. Table 9 shows examples of point predictions with prediction intervals and probabilities to be adult for different patterns of allocated stages per sex.

Applying the Bayesian model for age estimation, using the mean of the posterior distribution as point prediction rendered better results than using the trimmed mean or ML estimate. The mean absolute error was 2.0 years in females (median (Me) = 1.7, interquartile range (IQR) 0.8–2.7) and 1.7 years in males (Me = 1.6, IQR 0.6–2.5) based on the mean of the posterior distribution. The mean error was 0.1 years in females (Me = 0.0, IQR -1.7–1.8) and males (Me = 0.0, IQR -1.4–1.9). Root mean squared error equalled 2.38 (95% confidence interval (CI) 2.11–2.65) for females and 2.06 (95% CI 1.79–2.33) for males. Coverage of the 95% prediction interval was 94.7% (142/150) for females and 91.4% (107/117) for males.

Moreover, the error of the age estimate clearly depended on age. The dependency was lowest using the mean of the posterior distribution as point prediction with Spearman correlation (r) for mean error in females equal to 0.51 (95% CI 0.38–0.62, P < 0.001) and in males equal to 0.50 (95% CI 0.35–0.62, P < 0.001). For mean absolute error r = 0.10 in females (95% CI -0.06–0.25, P = 0.22) and r = 0.29 in males (95% CI 0.11–0.44, P = 0.0017).

Performance of the Bayesian procedure to discriminate between minors and adults is summarized in Table 10. In forensic age estimation in the living, one should strive for an approach with high specificity and NPV (specificity being the major concern). Estimating age based on the ML estimate rendered the highest specificity in females and males. The highest NPV was obtained using the mean of the posterior distribution as point prediction of age. The AUC was very similar for all three point predictions. The AUC probability to be older than 18 years was 0.869 (95% CI 0.811–0.926) for females and 0.948 (95% CI 0.908–0.988) for males.

Figure 11: Graphs comparing chronological age (dots) with the point (triangle) and interval (line) prediction in females (A) and males (B). The point prediction is the ML estimate.

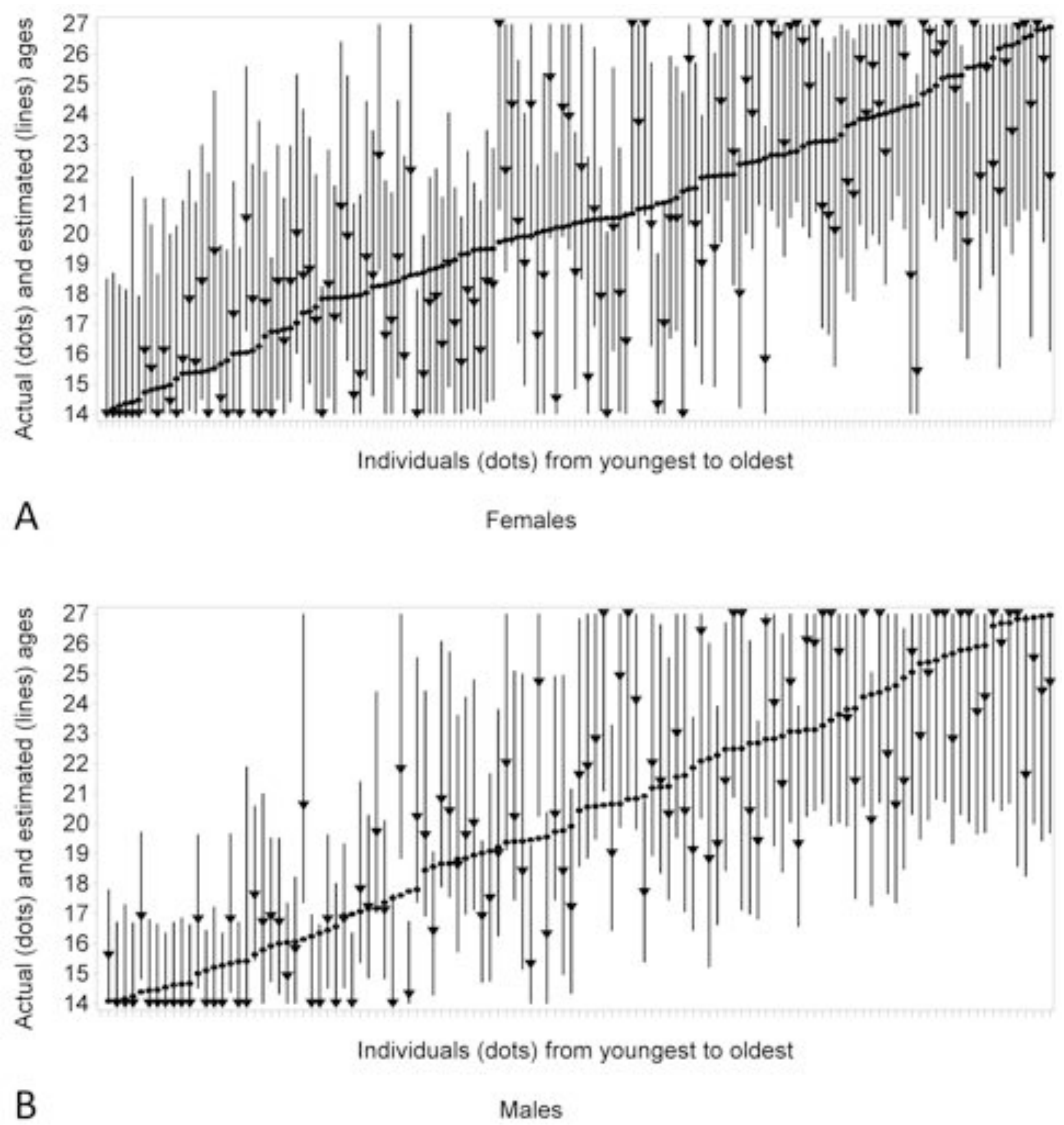


Figure 12: Posterior density for all possible homogenous stage patterns (same stage for all third molars) in males. When all third molars are in stages equal to or lower than three (3333), the distribution of age is right-skewed. This smoothly evolves to a left-skewed age distribution when all third molars are fully mature (8888). Around the age of 18 years, most individuals have third molars in stage 5. Per situation the probability to be adult is represented by the area under the posterior density curve to the right of the 18 years threshold (blue vertical line).

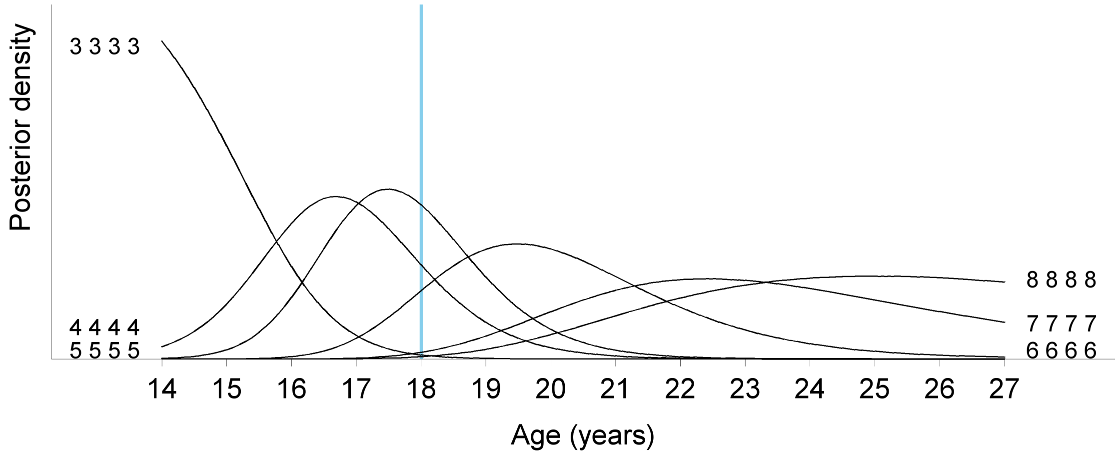


Table 9: Examples of point predictions with prediction intervals and probabilities to be adult for different patterns of allocated stages per sex. Point predictions of age are based on the mean of the posterior distribution. Notice that the lower limit of the prediction interval in the lowest stage reflects the minimum age in the study sample. Similarly, the upper limit in the highest stages equals the maximum age in the study sample.

Element 18	28	38	48	**Point prediction**	**95% Prediction interval**	**Probability to be adult**
				Females		
3	3	3	3	15.53	(14.00–18.33)	0.0600
4	4	4	4	16.89	(14.00–20.78)	0.2496
5	5	5	5	18.59	(14.35–22.74)	0.5760
6	6	6	6	21.88	(17.76–26.73)	0.9509
7	7	7	7	23.96	(20.15–27.00)	0.9956
8	8	8	8	24.68	(21.17–27.00)	0.9989
				Males		
3	3	3	3	15.03	(14.00–16.68)	0.0037
4	4	4	4	16.90	(14.35–19.45)	0.1765
5	5	5	5	17.75	(15.35–20.33)	0.3746
6	6	6	6	20.17	(16.79–24.08)	0.8883
7	7	7	7	22.85	(19.25–27.00)	0.9926
8	8	8	8	23.76	(20.04–27.00)	0.9976

Table 10: Performance to discriminate between minors and adults.

	Predicted age based on	ML estimate	Mean	Trimmed mean
		Rate (95% CI)		
Females	Accuracy	77.3 (69.8–83.8)	79.3 (72.0–85.5)	78.0 (70.5–84.4)
	Sensitivity	78.9 (70.0–86.1)	84.4 (76.2–90.6)	82.6 (74.1–89.2)
	Specificity	73.2 (57.0–85.8)	65.8 (49.4–79.9)	65.8 (49.4–79.9)
	PPV	88.7 (80.6–94.2)	86.8 (78.8–92.6)	86.5 (78.4–92.4)
	NPV	56.6 (42.3–70.2)	61.4 (45.5–75.6)	58.7 (43.2–73.0)
	AUC	0.865 (0.809–0.922)	0.873 (0.817–0.928)	0.874 (0.818–0.929)
Males	Accuracy	90.6 (83.8–95.2)	90.6 (83.8–95.2)	89.7 (82.8–94.6)
	Sensitivity	91.0 (82.4–96.3)	92.3 (84.0–97.1)	91.0 (82.4–96.3)
	Specificity	89.7 (75.8–97.1)	87.2 (72.6–95.7)	87.2 (72.6–95.7)
	PPV	94.7 (86.9–98.5)	93.5 (85.5–97.9)	93.4 (85.3–97.8)
	NPV	83.3 (68.6–93.0)	85.0 (70.2–94.3)	82.9 (67.9–92.8)
	AUC	0.950 (0.912–0.988)	0.949 (0.909–0.988)	0.949 (0.909–0.988)

Accuracy = proportion of correctly classified subjects; Sensitivity = proportion of correctly classified adults; Specificity = proportion of correctly classified minors; PPV = proportion of adults within estimated adults; NPV = proportion of minors within estimated minors; AUC = area under the ROC curve.

DISCUSSION

Staging third molars' development.

Considerations for developing a staging technique

Staging development of third molars has been developed based on radiographs. However, with the increasing demand of imaging for age estimation that doesn't use ionizing radiation[19], MRI is being studied for dental age estimation by several research groups.[6-10] Since the appearance of teeth on MRI differs greatly from that on radiographs, a mere extrapolation of radiographical stages seems inappropriate. After all, criteria for staging based on crown height and root length cannot be applied when the cemento-enamel junction cannot be identified unambiguously. Dedicated MR sequences, in which it is possible to differentiate between the hard dental tissues, have been developed.[20-22] However, their use is not common practice yet, rendering them unavailable for forensic purposes. Therefore, a universally applicable MRI specific staging technique for third molar development was proposed in this paper.

Some authors stated that predictions of crown height and root length should be avoided because, especially in third molars, dimensions are highly variable and unpredictable.[18] Predictions always imply subjectivity, while objective criteria for stages should be pursued. It has been reported that precision of the staging method might be reduced – i.e. compromise the feasibility to register all of the stages correctly – if thresholds between stages are based on predictions of lengths of tooth parts.[2, 23] Moreover, the fully developed crown height cannot be used to predict the future mature root length.[24] In the current study, it was considered inappropriate to include a stage in which MR root length would be at least twice MR crown height, because it was noticed that the roots of some third molars never reached this length, even when fully matured. In literature, it was also

stated that because of variability between second and third molars, predictions of third molar lengths should not be based on or compared with the dimensions of neighbouring teeth.[18] Therefore, only objective criteria were used in the proposed staging technique.
To differentiate between stage 5 and stage 6, one could check the tooth's eruption. In stage 5 it is still in infra-occlusion, while in stage 6 it has reached the occlusal plane. However, third molars are often impacted or they over-erupt (when they don't have an antagonising tooth), which makes these characteristics inapplicable. Therefore, eruption was not included in the criteria for MR specific staging.

Tooth development and assessability
It can be considered a limitation of the current study that both participants with and without third molar impaction, agenesis or extraction of other teeth were included to generate the model for age estimation. However, in the general population several patterns of agenesis/extraction/impaction are present and it is not feasible to take all different patterns into account for age estimation. It has been stated that agenesis and impaction might delay third molar development[11, 25-31], while extractions might accelerate it.[32, 33] It is our intention in future research to study this on MRI in the current study population.
In about 90% of upper third molars the palatal root was the least developed one, meaning it was either less developed or equally developed as the buccal roots. Also in about 90% of lower third molars the distal root was the least developed. These numbers are lower than reported based on a subset of the current study population (98% and 95% respectively).[10] In some cases not all roots could be assessed on MRI. This was more frequent in the upper jaw, mainly due to the small dimensions of the buccal roots, as previously reported by De Tobel at al. (2017).[10] Few studies reported on the relative development of different roots within the same third molar.[29] In any case, the least developed root should be considered, to grant the benefit of the doubt.
Baumann et al. (2015) mentioned that 5% (15/307) of molars could not be assessed, due to technical reasons (e.g. motion artefacts), equally distributed among upper and lower jaw. However, this also included first and second molars. In the study by Guo et al. (2015) 2% (13/530) of lower third molars could not be assessed due to due to insufficient image quality. In the current study slightly higher numbers of unassessable teeth were encountered with 5% (51/1096) of upper and 7% (70/1044) of lower third molars. Whether this should be attributed to the MRI scanner and/or MRI sequence used, could be subject of future studies. However, the used sequence proved to be the most suitable after a selection process in De Tobel et al. (2017).[9]

Essential plane
Regarding the plane in which slices are deemed suitable for stage allocation, De Tobel et al. (2017) reported that sagittal slices were essential in 99% of cases.[10] They contributed this to the anatomy of third molars. This corresponds with the current number of 94%. Coronal and axial slices were less frequently useful in the current study (4% and 0% respectively) than in the previous study (11% and 8% respectively).[10]

Statistical approach to age estimation

Reproducibility of staging
It has been stated that reproducibility depends on the staging technique.[34] Inter- and intra-observer agreement in the current study were similar to or lower than those reported in previous studies on third molar development, as seen on 3D imaging modalities (Table 11).[6, 7, 10, 35, 36] It appears that studies including a larger sample of staged molars had relatively lower reproducibility values. Possibly, more easy to stage cases ended up in the small subsamples, used for reproducibility calculations. Furthermore, one might expect that staging based on computed tomography (CT) would be more reproducible than based on MRI, since MRI is more prone to artefacts and is more influenced by surrounding tissue and motion. From Table 11 however, it is clear that staging on MRI shows similar reproducibility as on CT. Although the presented staging technique did not outperform the established techniques (developed on radiographs), one could question the suitability of the Demirjian technique, since it is based on criteria that cannot be visualised with MRI, and the Köhler technique, since it is based on predictions of root lengths which are highly variable.[18]
Although intra- and inter-observer agreement was high, a substantial proportion of

disagreement remained (Tables 7 and 8). Two striking observations can be made: two stage differences occurred and systematically a higher stage was allocated during the second staging session. Both observations might be explained by a learning curve. In the first session, more often when observers doubted about the appropriate stage, the lower stage was allocated. Possibly they were more confident during the second session, with the first observer being more confident than the other. However, since some degree of uncertainty remained, more divergent results were obtained, compared to the first session. The learning effect was present in both observers, although observer 1 was more experienced than observer 2. An explanation might be that although observer 1 had seen more teeth on MRI than observer 2 and he had staged some series of third molars on MRI and panoramic radiograph for previous research, he had never staged a series this large. As was stated by other researchers, more experienced observers generate more consistent results.[37-41] An alternative explanation for two stage differences was when the considered third molar was depicted over several slices. In those cases one observer might have been more conservative and allocate the lower stage because in most slices that seemed appropriate. By contrast, the other observer might have reasoned that the root was in a higher stage, incorporating the slice thickness. When age estimation is done in practice, it is wise to assess the images with at least two observers and allocate stages in consensus.

Finally, also calibration of the observers influences reproducibility. Both observers were from the same research group and were trained in a similar way. It would be useful to see which results would be obtained by an independent observer, e.g. someone from another research group. Anyway, future research is necessary to verify the reproducibility of the proposed staging technique.

The only way to eliminate inter- and intra-observer variability is to conduct automated age estimation. Urschler et al. (2015) reported promising results on automated age estimation based on hand and wrist MRI.[42] Whether or not this approach can be extrapolated to other anatomical structures, such as third molars, is subject of further research.[43]

Table 11: Reproducibility of staging third molar development based on 3D imaging modalities.

Reference	Imaging modality	Elements	Staging technique	Intra-observer agreement			Inter-observer agreement		
				Statistic		N	Statistic		N
Baumann (2015)[6]	MRI	All molars	Demirjian	-		-	Cohen's kappa	0.51	312
Guo (2015)[7]	MRI	Lower left third molar	Demirjian	Kappa	0.89	60	Kappa	0.83	60
De Tobel (2017)[10]	MRI	All third molars	Demirjian	ICC	0.94-0.97	48-50	ICC	0.85-0.94	44-47
			Köhler	ICC	0.96-0.97	48-50	ICC	0.86-0.95	44-47
Current	MRI	All third molars		Weighted kappa	0.80-0.89	350-379	Weighted kappa	0.76-0.80	899
				Agreement	0.66-0.79	350-379	Agreement	0.77-0.87	899
Bassed (2011)[35]	CT	Lower third molars	Demirjian	Kappa	0.949	25	Kappa	0.842	25
				Agreement	0.96	25	Agreement	0.88	25
Cantekin (2013)[36]	CBCT	Lower third molars	Demirjian	Kappa	0.896	70	Kappa	0.692	70

(CB)CT = (cone beam) computed tomography; ICC = intra-class correlation coefficient; MRI = magnetic resonance imaging; N = number of assessed molars.

Age estimation

Baumann et al. (2015) demonstrated that compared with staging on MRI, slightly lower stages were allocated to the same third molar on panoramic radiographs.[6] Guo et al. (2015) found that the minimum age for a fully mature 38 on MRI was 19.57 years for females and 17.77 years for males.[7] Consequently, it seems that a mature third molar 38 on MRI could act as a sign of adulthood in females. In the current sample minimum ages were not used to discern minors from adults. Instead probabilities were calculated using the Bayesian model. When homogenous stage pattern 8 is seen, it is highly probable that an individual is over 18 years old (99.89% in females, 99.76% in males). However, around the age of 18, most individuals will have third molars in stage 5 (Figure 12).

With a mean absolute error of 2.0 years in females and 1.7 years in males, age estimation based on MRI of third molars is less accurate than a similar approach based on radiographs which had an overall mean absolute error of 1.13 years (Me = 0.89, IQR 0.44–1.62).[14] Third molar stages of 2513 individuals were included in their Bayesian model. It can be expected that age estimation performance based on MRI would increase when the reference sample would be larger. However, third molars are not routinely scanned with MRI, so retrospective data collection is impossible. Since several research groups are gathering third molar MRI data prospectively, joining forces could generate a more robust age estimation model.

The performance to discern minors from adults was better for males than for females, with specificities of 96% and 73% respectively. The AUC equalled 0.873 for females and 0.949 for males. Based on lower left third molar staging on radiographs, Liversidge and Marsden (2010) reported a specificity of 96% (females and males combined).[44] However, they reported separate statistics for the different stages, since they did not apply statistical modelling to estimate age. In their study AUC was 0.904 (95% CI 0.889–0.919).[44] Based on staging all third molars on radiographs, Thevissen et al. (2010) reported that specificity ranged between 33% and 87%, without obvious better results for either sex, using country-specific data in a Bayesian model.[45] An AUC of 0.853 was reported in another paper by the same research group.[14]

Because of the inherent inter-individual variability of development, several anatomical structures should be incorporated into the ad-hoc procedure. It has been demonstrated that combining the information of several developing structures increases accuracy of age estimation.[46-52] However, when combinations are used for age estimation, appropriate statistical methods should be used. Simple regression will generate unrealistically narrow prediction intervals. Instead, a Bayesian approach has been demonstrated to be the most suitable statistical method.[16, 53, 54] In view of adding information of other anatomical structures to the ad-hoc procedure used in the current study, the upper age limit of the study population (26 years of age) was higher than in other studies about third molar development (25 years of age[44], 24 years of age[7], 23 years of age[6], 22 years of age[14]).

CONCLUSION

A mere extrapolation of staging techniques for third molar development based on radiographs to MRI was considered inappropriate. Therefore, an MRI specific staging technique was proposed. Reproducibility was similar to other staging techniques. Although embedding this technique into a Bayesian model for age estimation did not outperform established age estimation methods based on radiographs, it opens the perspective of combining developmental MRI information for age estimation. Other anatomical structures can be added to the used third molars model.

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The age estimation practice related to illegal unaccompanied minors immigration in Italy

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ABSTRACT

The migrants arrived to the Italian coasts in 2016 were 181.436, 18% more than the previous year and 6% more than the highest number ever since. An "unaccompanied minor" (UAM) is a third-country national or a stateless person under eighteen years of age, who arrives on the territory of the Member State unaccompanied by an adult responsible for him/her whether by law or by the practice of the Member State concerned, and for as long as he or she is not effectively taken into the care of such a person; it includes a minor who is left unaccompanied after he/she entered the territory of the Member States.

As many as 95.985 UAMs applied for international protection in an EU member country just in 2015, almost four times the number registered in the previous year. The UAMs arrived in Italy were 28.283 in 2016; 94% of them were males, 92% unaccompanied, 8% of them under 15; the 53,6% is 17; the individuals between 16 and 17 are instead the 82%. Many of them (50%), 6561 in 2016, escaped from the sanctuaries, thus avoiding to be formally identified and registered in Italy in the attempt to reach more easily northern Europe countries, since The Dublin Regulations (2003) state that the asylum application should be held in the EU country of entrance or where parents reside. The age assessment procedures can therefore be considered as a relevant task that weighs in on the shoulders of the forensic experts with all the related issues and the coming of age is the important threshold. In the EU laws on asylum, the minors are considered as one of the groups of vulnerable persons towards whom Member States have specific obligations. A proper EU common formal regulation in the matter of age estimation procedures still lacks. According to the Italian legal framework in the matter, a medical examination should have been always performed but a new law completely changed the approach to the procedures of age estimation of the migrant (excluding the criminal cases) with a better adherence to the notions and concepts of vulnerability and psychological and social maturity.

Migration of unaccompanied minors in Italy

In 2015, the European Union (EU) offices received a record number of more than 1.392.155 applications for international protection, a number which represents the highest ever with the sharpest year-to-year growth (+110 % compared to 2014) since 2008. Germany (34 % of all applicants), Italy, Sweden, Austria and Hungary are the EU countries that received the higher number of applications.[1] The migrants arrived to the Italian coasts in 2016 were 181.436, 18% more than the previous year and 6% more than the highest number ever since.[1] The interpretation of these already dramatic numbers becomes especially worrying if we consider that as many as 95.985 unaccompanied minors (UAMs) applied for international protection in an EU member country just in 2015, almost four times the number registered in the previous year. The UAMs arrived in Italy were 28.283 in 2016; 94% of them males, 92% unaccompanied, 8% of them under 15; the 53,6% is 17; the individuals between 16 and 17 are instead the 82%.[2] The largest part of the minor migrants comes from Nigeria, Eritrea, Guinea, Ivory Coast and Gambia. Many of them (50%) very soon go off the grid (6.561 in 2016), a huge number, rising from the 1.754 in 2015,[2] escaping from the sanctuaries, thus avoiding to be formally identified and registered in Italy in the attempt to reach northern Europe countries more easily, since The Dublin Regulations (2003) state that the asylum application should be held in the EU country of entrance or where parents reside. To be considered, however, is the fact that not any minor come to Italy unaccompanied and not any UAM then submits an application for asylum. The authorization to stay in Europe is not granted to illegal migrants if they do not obtain a specific asylum permission and if they are not unaccompanied minors.

The 50%, anyway a huge number, settle down in Italy, even if a relocation programme was politically agreed at EU level to support the frontline EU border member states – mainly Italy and Greece – that were, since the beginning of the migratory flows, under considerable social, economic and political pressure and struggling for the lack of financial resources.

International and EU legal framework

The following are the most relevant international and EU directives in the area of asylum and minors' protection:

(1) The Declaration Of The Rights Of The Child and the Convention On The Rights Of The Child

The first document, proclaimed by UN General Assembly Resolution 1386(XIV) of 20 November 1959 was the basis of the Convention of the Rights of the Child adopted by the UN General Assembly many years later on 20 November 1989 and entered into force on 2 September 1990. It stated in the prologue that "the child, by reason of his physical and mental immaturity, needs special safeguards and care, including appropriate legal protection, before as well as after birth". The focus on the notion of immaturity in this early document is therefore pretty clear actually anywhere. In the Principle 2 of the resolution it is highlighted that in the enactment of laws for the purpose of protection and development of the best conditions for the child, the best interests of the child shall be the paramount consideration. In the Principle 5, we find other important hints relying on the notion of vulnerability: "The child who is physically, mentally or socially handicapped shall be given the special treatment, education and care required by his particular condition" and again "Society and the public authorities shall have the duty to extend particular care to children without a family". The latter document was adopted by UN General Assembly Resolution 44/25 of 20 November 1989, in the thirtieth anniversary of the Declaration of the Rights of the Child, and entered into force on 2 September 1990. In art.1 it stated that "For the purposes of the present Convention, a child means every human being below the age of eighteen years unless under the law applicable to the child, majority is attained earlier". The 18 years age threshold has been then decided, despite any previous consideration of the notions of maturity and vulnerability.

No recommendations or regulations are included in the aforementioned documents about procedures to be adopted for the age estimation of the unaccompanied migrant.

(2) The UN Committee on the Rights of the Child, 2005, 2007

In General Comment No.6 on the Treatment of Unaccompanied and Separated Children outside their Country of Origin, the UN Committee on the Rights of the Child states that identification measures, including age assessment, should not take into account only the physical appearance of the individual, but also his or her psychological maturity. Moreover, the assessment must be conducted in a scientific, safe, child and gender-sensitive and fair manner, avoiding any risk of violation of the physical integrity of the child; giving due respect to human dignity. The Committee stresses that if there is no proof of age, the child is entitled to a reliable medical and social investigation that may establish his/her age and, in the case of conflict or inconclusive evidence, the child shall have the right to the rule of the benefit of the doubt and be treated accordingly.

(3) In May 2010, the European Commission presented an action plan for unaccompanied minors who are regarded as the most exposed and vulnerable victims of migration.
According to the Directive 2013/33/EU, Chapter 1 art. 2, of the EU Parliament and Council (26.06.2013) laying down standards for the reception of applicants for international protection, an "unaccompanied minor" is a third-country national or a stateless person under eighteen years of age, who arrives on the territory of the Member State unaccompanied by an adult responsible for him/her whether by law or by the practice of the Member State concerned, and for as long as he or she is not effectively taken into the care of such a person; it includes a minor who is left unaccompanied after he/she entered the territory of the Member States.
The very important threshold of 18 years is therefore determined, as far the coming of age - that is the attainment of 18 years of age - defines the individual as an adult, with all the inherent important implications on acceptance or refusal of the asylum application.
In the EU laws on asylum, the minors are considered as one of the groups of vulnerable persons towards whom Member States have specific obligations. Minors are entitled to a guardianship and their needs must be taken into account when implementing the provisions of the EU Reception Directive. The EU states must guarantee access to rehabilitation services to those who have been victims of any form of abuse, neglect, exploitation, torture or cruel, inhuman and degrading treatment or who have suffered from armed conflicts.[3,4]

UAMS identification procedures: age estimation

The task of correct identification of the minor is of huge importance as far as the detention of minors must be considered a matter of real concern as teenagers, especially if unaccompanied, separated from their family, are susceptible to mental and emotional distress, prone to self-harm and illegal behaviours.
The only way to perform a real, chronological age "determination" of the AS should be a documentary evidence; in all the other ways only an "estimation" is obviously possible. Unfortunately, however, it has been estimated that around 51 million births go unregistered each year in developing countries, mainly in South Asia and sub-Saharan Africa. Even when a birth has been registered, the individual may lose the documentation and have no way of replacing it, particularly in times of war or social unrest. Again, there is an unfortunate geographical coincidence of incomplete birth registration rates, wars and poverty which means that refugees and asylum seekers often come with no evidence of age.
The age assessment procedures can therefore be considered as a relevant task that weighs in on the shoulders of the forensic experts with all the related issues.
A proper EU common formal regulation in the matter of age estimation procedures still lacks. The procedures for age estimation and requirements to be performed at a continental level, are currently likely governed only by simple technical recommendations, mainly issued by national or local scientific institutions. 23 EU countries use carpal (hand/wrist) X-ray; 17 countries, dental X-ray; 15 countries, collar bone X-ray (over 18); 14 countries, dental observation; 8 countries use sexual maturation observation; 2 countries rely on a psycho-social assessment only.[5]
Such a plurality of approaches rises ethical problems regarding to the migrants' right to be correctly informed about the procedures to follow, properly assessed with a ratified homogenous and shared protocol, issued according to the most recent and accepted scientific evidence.
No EU law or regulation currently rules the standard of evidence legally required in age assessment decisions (on the balance of the probabilities vs. sure of the necessary facts). Everything is therefore devolved to the national interpretation of the ethical principle of beneficence according to which any decision must be anyway taken in the minor's best interest.
No agreement has been reached so far in the EU about the possible ethical and legal justification of methods of analysis of bone and teeth maturation relying on the use of X-ray exams, given that the medico-legal assessment of age cannot definitely be described as a clinical diagnostic procedure.
But even if we temporarily overcome the aforementioned special problem of justification of protocols and procedures, we should spend some words about the scientifically proven accuracy of the adopted approaches. Three main

approaches are currently adopted: a simple physical assessment approach, which relies only on physical and skeletal maturation evaluations; a pure psycho-social assessment; and an holistic one, which keep into consideration not only the result of the body physical examination but also those of the skeletal and teeth X-ray examination and a psycho-social assessment.
Just as a reminder, the UN Committee on the Rights of the Child in its General Comment on the Treatment of Unaccompanied and Separated Children outside their Country of Origin stated that age assessments "should not only take into account the physical appearance of the individual, but also his or her psychological maturity, interaction of person with the interviewer, social history and family composition, developmental considerations, education, independence and self-care skills, general health and medical conditions, information from documentation and other sources", therefore a real holistic procedure.
According to the Italian legal framework in the matter, a medical examination should have been always performed so far, but a new State law[6] completely changed the approach to the procedures of age estimation of the migrant (excluding the criminal cases) with a better adherence to the notions and concepts of psychological maturity, interaction skills, social history, education, vulnerability, independence and self-care and coping skills, cited above. This new law, which privileges in the first place the administrative and documentary age determination and the psycho-social interview, relegates any medical procedure of assessment as the last tool, to be adopted only in case of persistent and founded doubts about the age of the migrant.

Age assessment

It is beyond the aim of this paper to summarize the vast literature existing about age estimation based on skeletal and dental maturation and relying on X-rays examinations but it is worth the effort to spend a couple of words about that since it is actually the main task for the forensic examiner.

Dental age assessment

Even if we realize that there is not a complete agreement in the literature about the correspondence of the different steps of the third molar radicular maturation and age, there is substantial agreement to consider an adult any individual at the last stage of maturation of the third molar radicular apex. No dental methods or parameters can be adopted to estimate age after the complete third molars root formation; generally speaking the stages of calcification of the third molar roots is a less regular as a phenomenon than that of the other teeth. Therefore, the study of the third molar development is more useful when not an age estimation but rather a classification of age with respect to the 18 years age threshold is requested.[7-22]
Moreover, a mature image is informative (at least 85-90% of probabilities of the attainment of 18 years) while the image of an immature apex is not, and this could lead to a large proportion of adults being assessed as minors. Moreover, Rodrigues Barros Soares et al. disagree stating that a full maturation of the third molars is found in the age average of 17.9 years, admitting therefore the possibility that a minor could have fully developed third molars.[23] The UK Royal College of Paediatrics and Child Health (RCPCH) said about dental age assessments: *"There is not an absolute correlation between dental and physical age of children but estimates of a child's physical age from his or her dental development are accurate to within two years for 95% of the population and form the basis of most forensic estimates of age"*.

Skeletal age assessment

The Literature indicates that Greulich and Pyle (GP) is the most widely adopted method for skeletal age assessment, a method which was introduced into practice exclusively for auxological studies and that was only later adopted for forensic purposes and procedures, and derived by the analysis of few Caucasian North-American middle class individuals living in the past century. Regarding the skeletal methods, it has to be considered also that many conditions can affect skeletal growth, such as nutritional/ metabolic, socio-economical, pathological (major effects of the endocrine diseases on bone development and growth, such as precocious puberty, adrenogenital syndrome, hyperthyroidism), genetic alterations. Regarding the influence of ethnicity, we can say that no agreement has been reached in the literature yet, even if many authors consider ethnicity an important factor affecting growth. According to Schmeling A. et al., however, ossification is mainly influenced by the socio-economic status of the considered population other than by ethnic considerations: the lower the status, the higher the risk of underestimation of growth/age.[24-27] Many authors indeed agree to consider the necessity of the adoption of ethnic-specific adjustment factors in the use of the skeletal age methods because ethnic and racial differences in growth patterns exist at specific ages.[28-29] It must be considered that most UAMs come from poor developing countries where patterns of maturation are likely to differ either from a genetic/ethnic or a socio-economic/nutritional point of view. The potential margin of error intrinsic in the use of a skeletal examination gets even bigger when no news are available about the individual's clinical history. According to the Literature, the accuracy of the skeletal methods for age estimation is poor in the age range 16-18, with a high percentage of overestimations; the left hand/wrist study is then completely useless over 18 years.[30] In any case, however, the estimation provided with the skeletal methods is everything but individually tailored to the proband and greater attention should be otherwise put to inter-individual variability.

Psycho-social interview

The psycho-social interview, in fact, even if on one hand it can be largely influenced by many biases such as the historical, political and social context in which the assessment is being made, the poor and unfamiliar setting in which the assessment is done, the lack of scientifically determined data about the overall margins of error, on the other hand it anyway seems the more suitable method to catch all the maturity and vulnerability aspects of the personality of the migrant, overcoming any strict limitation imposed by an age threshold.

All that said, we must admit that there is no method (neither medical or non-medical) available so far that enables the assessment of chronological age to the accuracy requested by government and border agencies, especially around the critical age threshold of 18 years; it is easier indeed to estimate the compatibility of the declared age with the 18 years of age threshold.

What happens in Italy?

In Italy the UAMs are under the full administrative responsibility of the City Council that is in charge also of the daily expenses for the minor - about 35 euro per day - which is about as much as twice the amount paid for any adult migrant. But, despite the high numbers of migrants arriving in the Italian coasts and the consequent costs, very few age assessments are eventually performed. All of them are requested by the Government local offices, but ordered by the Public Prosecutors (PP) at the local Magistrates' Court offices.

Generally speaking, the age assessment in Italy is requested mainly in case of unaccompanied migrants or criminal proceedings. It has been performed, at least until April 2017, with a skeletal (left hand-wrist X-ray, LHW) and sometimes also dental (ortopantomographs) development data analysis. In the cases dealing with illegal migration, however, the best practice – with the combination of skeletal and dental data analysis, as it is recommended in the Literature - is followed indeed only in few cases.[31,32] The skeletal assessments are often performed with the Greulich and Pyle (GP) method by Accident & Emergency (A&E) radiologists without any experience of age estimation cases and who have not received any specific education in the field. They often provide assessments that lack of any description of the margins of error or percentage of possible misjudgement with respect of the 18 years threshold.

No data are available on the frequency of adoption of physical and sexual maturation examinations for age assessments of minors in Italy, methods poorly accurate and no more acceptable also for ethical reasons. Physical and sexual examinations in fact are highly questioned both for being scarcely accurate and an humiliating practice for children that sometime have already experienced sexual violence, exploitation or ritual mutilations of genitals.

Even if in a substantial lack of real data, we can say that the physical examination of the minor is adopted in very few cases in Italy also because the assessment is mainly performed only with X-rays by the A&E doctor who is not qualified and competent indeed for any other kind of examination.

In Italy, the widespread practice to estimate the age or to provide a classification with respect to the 18 years threshold only with the X-ray of left hand-wrist implies a great risk of misclassification and overestimation considering that a mature LHW can be found even in a minor. On the other side, the teeth might provide useful

evidence with the third molar full maturation, tooth which seems not influenced by ethnic and environmental factors, but a dental assessment very seldom is required by the PP.
According to the Italian legal framework in the matter, a medical examination should have always been performed so far, but a new and recent law[6] completely changed the approach to the procedures of identification and even age estimation of the migrant (excluding the criminal cases), with a better adherence to the notions and concepts of psychological maturity, interaction skills, social history, education, vulnerability, independence and self-care and coping skills. This new law, which privileges in the first place the administrative and documentary age determination and the psycho-social interview, relegates any medical procedure of assessment as the last tool, only in case of persistent doubts about the age of the migrant.
According to the new law, the documentary evidence must be firstly pursued with the consent of the minor, even if this would require to activate the diplomatic routes. The age assessment is requested only if absolutely necessary and not systematically, in respect of the minor's right to preserve his/her identity, and preserve from any possible negative psychological and emotional outcome in case of doubts about identity and age.
The age assessment performed with the analysis of the narrative data retrieved from the UAM interview and a psychological evaluation of the UAM's demeanour, other than the attainment of a specific chronological or biological developmental stage, tends mainly to determine the attainment of the individual's level of maturity, which is ultimately the understanding if the individual can be considered able to successfully look after her/himself and cope with the everyday life's tasks eventually in the new host country. Hence we must consider maturity and vulnerability result in an inversely proportional ratio. Any procedures of age assessment based on the evaluation of the attainment of a maturity level can be defined as "needs-oriented".
The psycho-social assessment, performed - according to the new Italian law - by experts in a neutral setting, is one of the mainstays of the age estimation procedures defined by the new law. This is a method that more accurately than ~~others~~ accounts for the evaluation of the young migrant's real vulnerability or psycho-social autonomy, a principle which opens the door to an important discussion about what is a vulnerable person and if an age threshold is the best way to define it. The focus of the Italian law on the minor's vulnerability and protection is therefore better represented in this psycho-social approach, despite any consideration about the scientific basis of the assessment and the possibility to indicate a margin of error of the estimation. The medical methods of assessment (included the odontological) are therefore currently relegated to a second stage. The medical assessment must be performed with a holistic approach by specialized personnel in a proper setting. No exams which can be potentially invasive or harmful for the minor's psycho-physical condition can otherwise be performed. The margin of error of the estimation must be always clearly indicated in the report. The minor age is always presumed if any doubt still persists.

Very often the migrant arrived in Italy lies to the officers regarding his/her real age or sometimes changes his/her mind just once arrived in the sanctuary. Sometimes lies are different from any expectations: (1) The migrant claims to be minor but he/she is not. This is the typical situation when age estimation is requested in criminal cases. The young criminals know that the punishment, if any, is decided according to juvenile Laws. So asylum seekers are sometimes prone to declare a lower age to be admitted to receiving international protection as refugee unaccompanied minors. (2) Nevertheless we were involved instead in a few cases of children that declared an older age in the aim to avoid the guardianship controls which International law requires. In these cases the migrants think that being considered an adult would provide them with more freedom of movement. Sometimes the adult migrant who declared to be a minor changes his / her mind once arrived at the sanctuary, when he/she realizes that the guardianship offered to the minor reduce any possibility of movement and full personal autonomy.
In these cases the pure chronological age estimation showed off all its limits in assessing the maturity and vulnerability of the migrant.
We had recently to face the explanatory and sad cases of two migrants, both in the age range about 18 but classified as adults, who despite any

considerations about age showed a fatal, tragic and deadly lack of psycho-social maturity.
One of them, fled off the grid of his sanctuary, trying to reach northern Italy or perhaps northern Europe. His social immaturity made him take the deadly choice to follow the train trucks and enter a completely dark tunnel dedicated to high speed trains, 32 km long, which he never saw the light again from.
Another migrant of the same age range, adult for the officers, bypassed the institutional limitations and went in the middle of a forest, where he lost his way and decided to put an end to his desperate escape hanged to a tree.
The cases show that a mere assessment of the biological age does not enable to catch the individual's level of maturity, which is ultimately the understanding if the individual can be considered able to successfully look after her/ himself and cope with the everyday life's tasks in the new host country, quite different from those of the country of origin. A more needs-oriented assessment might therefore be considered most suitable or even necessary, as part of a holistic approach.

CONCLUSION

Even if a more scientific and formal protocol should be applied in the age assessment of the migrants, things seem to go quite different in the daily practice.
Many migrants lie to the officers about their real age. It could be likely expected that many declare to the officers to be minor just to make the acceptance in the EU easier but the opposite really often happens instead. Many minors, in fact, try to be considered adults in the attempt to skip the restrictions of the compulsory guardianship attributed by law and the consequent limits to the personal freedom of movement or trying to reach another country in northern Europe.
An important reflection spontaneously rises, however, when we consider the attempts of the minors to escape the guardianships and the limits to the freedom of movements imposed in the sanctuaries. This issue, in our opinion, deals with the notion of a sort of social maturity much more than the implicit possession of all the sort of skills at the chronological attainment of the coming of age. Thus – in our opinion – the estimation of a chronological age gives poor results and shows its insufficiency in the evaluation of the needs and the vulnerability of the migrant, which are, basically, the most important notions to consider and appraise according to the Declaration Of The Rights Of The Child and the UN General Comment No.6 on the Treatment of Unaccompanied and Separated Children outside their Country of Origin.
The two reported cases, very sad stories of social disability, vulnerability and immaturity, made us think that the new Italian law, more committed to catch any possible need and any sort of vulnerability of the young migrants - actually the first in the EU in which a protocol for the migrants' identification is stated – adopts the procedure most adherent to the founding notions of solidarity originally included in the Declaration Of The Rights Of The Child and the UN General Comment No.6 on the Treatment of Unaccompanied and Separated Children outside their Country of Origin.

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Forensic odontology education: from undergraduate to PhD - a Brazilian experience

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ABSTRACT

Background: Forensic Odontology is a topic present in the majority of Dental Schools in Brazil, and due to this reality, some universities develop activities related to undergraduate and graduate students, from the Dentistry course until the Ph.D. degree.
Aim: To present the education experience related to Forensic Odontology at School of Dentistry of Ribeirão Preto (USP - University of São Paulo), showing the strategies and activities in the different degrees (Dental course, Forensic Odontology Specialization Program, Specific Professional Training, Master, and Ph.D.).
Results: To the undergraduate students, many activities are developed in order to demonstrate all the possibilities that Forensic Dentistry allow, including theoretical and practical activities; in the Forensic Odontology Specialization Program, the dentists are trained to act as Forensic Odontologists in all its amplitude; in the Specific Professional Training, some courses are available, related to specific topics as DVI, Forensic Facial Reconstruction, Auditor in Dental Care Insurance and others; and in the Master and Ph.D. Programs, the professionals receive training in skills like teaching, research, student's guidance and others.
Conclusion: In Brazil, Forensic Odontology is a well-known field in Dentistry and universities develop an important role in training a qualified workforce.

INTRODUCTION

Higher education in all countries is essential in human development and social mobility, not only training specific skills but also become professionals able to act according to ethical precepts.[1]
After the Brazilian Decree 3860/2001,[2] education institutions have to be divided according to their academic organization into: universities, colleges and integrated colleges, institutes and universities, technical education centers. Universities should follow the principle of indivisibility of teaching, research and extension, which differentiates them from other institutional forms of higher education.[2]
All the Brazilian universities have autonomy to create and arrange courses and graduate programs[2] and, accordingly, the expressions *lato sensu* and *stricto sensu* were introduced into the

education system to differentiate graduate courses, where *lato sensu* comprises specialization programs and *stricto sensu* are master's and doctoral programs, but in both, open to candidates that concluded an undergraduate course[3], and according to the specific area.
In 2001, several higher education programs in Brazil were modified, and Dentistry was not different. National curriculum guidelines in Dentistry are arranged in the Resolution CNE/CES 03/2002 which, in Article 6, paragraph II appears that one of the essential elements for the degree course in Dentistry should be human and social sciences, including knowledge regarding legal and ethical issues, and contemplate the complementary activities that higher education institutions should have, were scientific research and extension programs are placed.[4]
The interest in Forensic Odontology in Brazil[5] and in the world[6] is growing, but there is still low workload for the discipline and a small number of qualified professors in this specialty.[5] Outside the country, one of the main reasons for the lack of knowledge about forensic dentistry discipline is because it is not part of the mandatory content in the curriculum of undergraduate courses in Dentistry, and yet there few graduate courses in this area.[6]
The objective of this research is to prove the educational experience of the Forensic Odontology branch at School of Dentistry of Ribeirão Preto (USP - University of São Paulo), showing the strategies used and the different activities of the different degrees (Dental course, Forensic Odontology Specialization Program, Specific Professional Training, Master, and Ph.D.).

RESULTS

Undergraduation

As recommended by the Pedagogical Political Project[7] of the Ribeirão Preto Dental School, University of São Paulo, Forensic Dentistry looks for the integral formation of the student by linking education, research and extension. Upon graduation, many activities are designed to show all the possibilities that the Forensic Dentistry offers, including theoretical and practical activities through three different subjects: (1) Bioethics and Professional Ethics, taught in the 1st semester; (2) Dental Law, which takes place in the 8th semester; and (3) Forensic Dentistry, during the 9th semester.
The practical activities developed during the under graduation imply experience in several fields of activity of the dentist at the interface with the legal and forensic activities, (Figure 1) including archaeology and forensic anthropology, where students are placed in front of a situation in which they must carry out digging procedures to search for skeleton remains, and later, compose the anthropological report and perform identification by dental methods. The activity of forensic trauma involves the description of specific lesions, exposed in dental mannequins. In the dental office activity, students must during the semester make all necessary documentations to open a dental office in their cities, as well as a blueprint showing the dental office organization. Continuing the graduation activities description, in the crime scene exercise the students are faced with the reproduction of a crime scene, and should analyze the context and write a report with photos and a hypothesis of what happened.

Graduation

In the *stricto sensu* graduation course, the Forensic Odontology area has master's and PhD students, who receive training in skills like teaching - through seminars, didactic and pedagogical training, as well join the professor in the activities during the Dentistry degree course; Research - supervising scientific research, preparing their theses, writing scientific papers; and Extension - monitoring the expert routine developed in collaboration with the São Paulo State Court, in civil expertise cases and in association with the Legal and Medical Centre in forensic cases involving anthropological examination and human identification in Forensic Dentistry.
In the *lato sensu* graduation course, the Forensic Dentistry specialization aims to form a Forensic Odontology specialist, with extensive theoretical and practical hours, where they receive training and skills to act and work in all fields related to Forensic Dentistry. The practical activities (Figure 2) involve all these different fields allowed in Brazil: civil, criminal, labour and administrative lawsuits, contemplating knowledge in different fields of work: expertise in civil matters, dental records, forensic ballistics, archaeology and forensic anthropology, dental audit, forensic facial reconstruction, forensic genetics, traumatology, thanatology, crime scene investigation, expertise in labour suits, mass disasters, Lawental law and ethics, training of expert services, age

Figure 1: Practical and expository teaching activities carried out in the undergraduate course, USP - Ribeirão Preto Dental School, forensic dentistry area.
(A) and (B) Images concerning the practice of forensic archaeology.
(C) Practice of human identification by dental records.
(D) and (E) Forensic traumatology activity with the production of traumatic injuries in dental mannequins and volunteers.
(F) Crime scene investigation practice

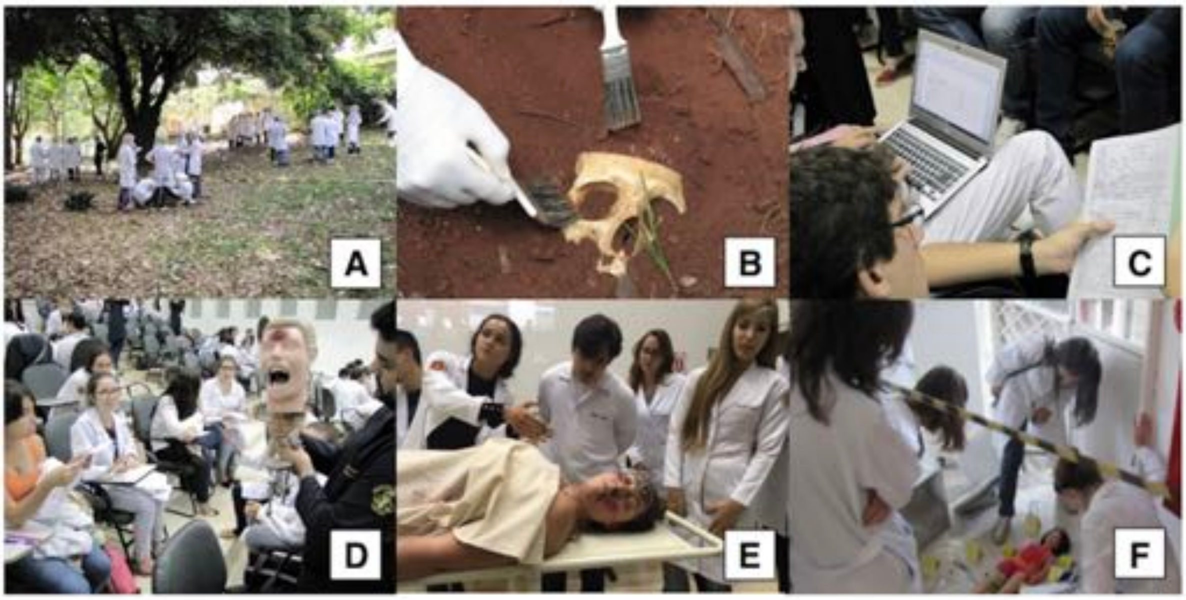

Figure 2: Practical activities carried out in the Forensic Dentistry Specialization Course, USP - Ribeirão Preto Dental School.
(A) Age estimation by different dental methods.
(B) Forensic anthropology practical activity.
(C) Forensic archaeology.
(D) Forensic ballistics.
(E) Practical activity, crime scene investigation.
(F) Human identification by dental records.

estimation, bite mark, writing reports, documentoscopy, forensic photography, forensic toxicology and many others. This level of training, initiated in 2010, already has about 100 qualified professionals to work in Forensic Dentistry.

Extension program

Related to the university extension program, several activities are developed, always in the interface to other education levels (undergraduate and graduate): 1) Civil and criminal expert routine; 2) Forensic Odontology College Extension Program; 3) Formation Course for Dental Auditor; 4) 3D Digital Forensic Facial Reconstruction Course; and 5) Mass Disaster identification Training Program.

1) The performance of expertise in civil matters takes place in collaboration with several districts of the São Paulo State Court, in which most cases are related to dental malpractice litigation, adding more than 90 records over the last five years. In the criminal court, the action takes place in partnership with the Forensic Anthropology Laboratory of the Centre of Legal Medicine (CEMEL) at USP - Ribeirão Preto Medical School, performing human identification and contributing for forensic anthropology exams.

2) Forensic Odontology College Extension Program has trained over 30 professionals, and this program aims to show the professional activities in the area, living and becoming familiar with a range of experiences. This project includes follow-up activities of the undergraduate courses, participation and contribution in the execution of forensic procedures, preparation of scientific papers and organizing events and activities in the routine of the Forensic Odontology team.

3) The Formation Course for Dental Auditor is offered as an immersion course, with a workload of 48 hours and six-day, which aims to enable the dentist to work in the dental insurance companies. This activity has trained more than 150 dental auditors in ten different groups.

4) The 3D Digital Forensic Facial Reconstruction Course is offered annually as an immersion course (32 hours) during four days, in order to provide training in the practice of computerized facial reconstruction, enabling the student to create three-dimensional models and complete the digital sculpture process using a software. This activity has trained 18 professionals in its editions.

5) The Mass Disaster Victim Identification Training Program is a course conducted in partnership with the Federal Police Department and the National Police Academy, to create official experts trained in Dentistry to act in managing mass disaster. The event, which has two complete editions and more than 40 experts prepared, was previously initiated to comply with the safety requirements of the largest events held in Brazil – the World Cup and the Olympic Games. The training includes a theoretical and practical approach to implementation of INTERPOL protocols for Mass Disaster Victims Identification in all its stages (*antemortem, postmortem* and comparison) including the simulation procedures.

DISCUSSION

Undergraduation

Forensic Dentistry is the specialty that aims to research psychic, physical, chemical and biological phenomena that can reach or could have reached a man, alive, dead or bones, and even fragments or traces, resulting partial or total reversible or irreversible injury.[8]

In Brazil, there is no specific law obliging Brazilian universities to implement courses in their curriculum. There are curriculum guidelines that define the professional profile to be reached.[4] The national curriculum guidelines, related to Dentistry, defined that the graduate student must have in the constitution of their professional profile an approach to ethical and legal issues acquiring, thus, pristine moral education, together with the technical and scientific backgrounds.[4,9] Considering that the dental area closely related to these topics (ethical and legal) is Forensic Dentistry, it is possible to see how many Brazilian universities have adapted it in their curriculum in search of a better approach to the content and training of their students.[8,9] In other parts of the world, such as Norway, Australia, Malaysia, Japan, Nepal and some Latin American countries, even if in a different way, the Forensic Dentistry is also approached in universities, emphasizing its importance in teaching.[10]

According to the Resolution CFO-63/2005, the areas of expertise in Forensic Dentistry include human identification, civil, criminal, labour and administrative expertise, forensic thanatology, writing records, reports and opinions, forensic dental traumatology, forensic ballistics, expert routine in living, dead, intact or in fragments,

expertise in related traces, including spots or liquids from the oral cavity or in it, imaging for expert purposes, dental law, professional practice guidance and images exams in forensic purposes.
And, based on the above fields, after a meeting between the Brazilian Association of Dental Education (ABENO) and the Brazilian Association of Forensic Odontology (ABOL), a document was prepared which contained suggestions related to the subject "Teaching in Forensic Dentistry for the undergraduate level", as the hours to be given, period to be carried out, indicating that the content of the course should be divided into two periods, with minimum workload of 60 hours each, the first, Ethics and Law in Dentistry, conducted the onset of clinical disciplines; the second, Forensic Odontology, to be conducted over periods of graduation.[11] And in that same document it is suggested that the relevant topics to the specialist in Forensic Dentistry should be lectured: (I) Dental Code of Ethics and Ethical Process Code; (II) Law 5081/66; (III) Professional exercise (legal and illegal); (IV) Professional Liability; (V) Consumer Protection Code and its relationship with Dentistry; (VI) Dental Records; (VII) Professional fees; (VIII) Professional Confidentiality; (IX) Law applied to Dentistry; (X) Opening and installation of dental development in regard to the ethical and legal aspects; (XI) Practical activities relating to the content taught, (XII) Historical evolution of Forensic Dentistry; (XIII) Expert routine; (XIV) Human Identification; (XV) Forensic Traumatology; (XVI) Forensic Thanatology; (XVII) Forensic Anthropology; (XVIII) Labour accidents and diseases in Dentistry; (XIX) Forensic Genetics; (XX) Forensic Ballistics; (XXI) Bite marks; (XXII) Practical activities.[11]
In the Pedagogical Political Project (USP – School of Dentistry of Ribeirão Preto)[7] it is possible to find the objectives of all disciplines, as well as a brief table of the same, where it is verified that the disciplines, during the graduation, have the existing considerations in both documents mentioned.[7-9]
The practical activities during the undergraduate course aim to awake the student interest, allowing experience, even in a simulated way, the above content in the classroom. A similar crime scene practice developed in this school is performed at the São Paulo Dental School (USP) and, according to the authors; the pedagogical dynamics become the crime scene lesson well remembered for presenting related content with the knowledge acquired throughout the course.[12]
An important aspect of teaching forensic dentistry in undergraduate courses is to enable dentists to assist in forensic investigations if and when the need arises.[7-9] It is possible that the professionals are required to work in cases involving human identification by dental confrontation, disaster victim identification, age estimation and criminal cases involving bitemarks.[9]
For Alberti et al.,[13] the teacher's concern with the work methodology is fundamental for the planning of learning situations that involve the performance of activities that are related to work practice becoming essential at all education levels. Thinking about this improvement in the quality of the teaching-learning process, Forensic Dentistry develops several practical activities in different ways, trying to connect the theoretical content with the field of action of the Forensic Dentist.

Graduation

The existence of *stricto sensu* graduate program linked to this area, has the importance of investing in the training of skilled professionals to work in teaching the disciplines that include this specialty.[9] The participation of students in teaching, research and extension, as recommended by the pedagogical political project of the Faculty,[7] seeks to bring the reality of the teaching activity to the day to day of masters and doctoral students and thus acts preparing teachers and professionals who will assume future responsibility for continuing teaching this area.[11] Qualified higher education plays an important strategic role in the country's development, institutions and people.
At the same time that the main objective of the training is to act in academic life, it is considered as an opportunity for transformation and a great contribution to professional improvement.[14] However, despite the importance of the masters and PhD candidates, already evidenced previously, degree course in Forensic Dentistry is still little found within the structure of the Faculties of the country.[9]
In addition to the *stricto sensu* activities, there is also a *lato sensu* graduation course that aims to train the future Forensic Odontology specialist. This course seeks the extensive professional

training in order for the professional to work in different fields of knowledge, such as a Forensic Odontologist or Criminal Expert[15] needing, thus, a vast training. Currently, we can find around 600 specialists in Forensic Dentistry in Brazil, and they work in the civil area, as much as in criminal, labour and administrative office, representing a small number in the labour market close to the real needs of the country. Faced with this problem, it is evident the importance of the *lato sensu* graduate programs in Forensic Dentistry, training qualified human resources, able to act and respond to the existing demand in the Brazilian reality.[16]

Extension program

Finally, following the constitutional principle of the indissolubility of the three university dimensions,[7] Forensic Odontology (USP – School of Dentistry of Ribeirão Preto) also develops practical activities, which aim at the professional improvement through university extension programs. These extension programs are, briefly, the interaction between the university and society,[16] which is essential as it puts the human resources inside the Brazilian reality, bringing greater effectiveness in solving community problems.[17] In addition, extension practices are linked directly with the formative education because, in a satisfactorily functional university triad, there is mutual encouragement between its components.[18,19]

Dental auditor training course

Subsequent to the continuous rise in the number of newly signed contracts in the strictly dental segment, under the health insurance companies market,[20] the Dental Auditor Training Course was created in order to generate human resources in this field. Auditing is a traditional inspectional tool that monitors management systems, and when applied to Dentistry, it is a work focused on improving the quality of service, through standards and indicators previously established, making critical and ethical analysis of the provided dental assistance, both in the public (National Audit System - SUS) and the private sector (health insurance providers).[21] Furthermore, it is a mandatory activity, by law[22] as well as the rules of the National Regulatory Agency for Private Health Insurance and Plans (ANS)[23]. Additionally, auditing is one of the expert skills in Forensic Dentistry.[8] Thus, the professionals become able to work inside the private health insurance companies, which have recently grown significantly in coverage.[24,25] Similarly, strictly dental health companies, where there is the essential role of the auditor, follows this notorious expansion.[26]

Civil and criminal expert routine

The growth of malpractice claims for professional liability against dentists in Brazil follows a global trend, registering an increase of over 380% of litigations in 10 years[27] and, since the judiciary power does not hold all of the expertise that these lawsuits require, there is the need for the dentist acting as an expert in solving these hindrances.[28] Regarding civil and criminal expertise, the objective is to approach the university's technical and scientific resources with judicial and legal demands of these spheres, ensuring the purpose of the extension practice, which is to serve real needs of society.[29]

3D digital forensic facial reconstruction course

Nowadays, forensic facial reconstructions are a very useful tool in the human identification process, when applying traditional methods are not effective, or when there is little evidence about the deceased's identity. The technique is used in the attempt to create an approximation of the facial appearance of the individual, thus stimulating recognition so that then, in the presence of a suspect, identification is conducted with ease. Software had been dominating increasingly in this ground, making the process more agile, standardized and thus more effective.[30] Hence, due to the deficit amidst trained Brazilian experts to execute this technique, the Forensic Odontology (USP – School of Dentistry of Ribeirão Preto) alongside with Ebrafol (Brazilian Team of Forensic Anthropology and Forensic Dentistry), planned to contribute in the training of qualified human resources to implement the forensic facial reconstruction technique through the use of open-source software. Employing this technique at the national criminal forensic institutes brings undoubted benefits to the service provided to the population.

Mass disaster identification training program

Lastly, realizing the lack of education and training in the police forces against unexpected and calamitous events, the Disaster Victim Identification Training Course was the solution found to put together the efforts of the criminal expert teams in Brazil. The Federal Police Department (DPF) and the National Police Academy (NPA), in a partnership with USP – School of Dentistry of Ribeirão Preto, institutes the training course, which introduces the INTERPOL DVI GUIDE Protocol,[31] a world reference in this type of disaster management. This guide has been applied and perfected on previous experiences, such as the tsunami in Asia (2004); the crash of Air France Flight 447 (2009) and also the earthquake followed by tsunami in Japan (2011), where very satisfactory results were obtained in the identification of victims,[32] highlighting the importance of its guidance.

CONCLUSION

In Brazil, Forensic Odontology is a well-known field of Dentistry, and universities play an important role, training and qualifying professionals in this field. Along with the elucidation of the realized activities, it is clear that USP – School of Dentistry of Ribeirão Preto offers a complete training in all different levels within the forensic dentistry scope, ensuring full educational process.

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Day to day issues in the forensic identification practice related to illegal immigration in Italy

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ABSTRACT
The migratory flows to Europe from the African countries, Asia and Middle East, have hugely intensified in the recent years. In 2016, more than 98,000 out of a total of 260,000 migrants across the Mediterranean Sea arrived in Italy and in May 2017, the trend of arrivals is: Italy +576%; Greece -39% compared to previous years. Some migrants die before touching the sole of the European continent, during the crossing, often afforded with ships, made available by unscrupulous smugglers or criminal organizations, which are unsuitable for this type of transportation. The tremendous occurrence of migrant casualties during the Mediterranean Sea crossing remains underestimated and nobody, country officers or databank, can provide a reliable number of dead bodies in such a large and now, endemic phenomenon. Forensic officers, who intervened to examine migrants' corpses, are ideally required to perform the usual activity and to answer the routine questions about the causes of death by detecting signs of possible crimes and body identification. In practice, several specific issues and limits challenge the activity of the forensic professionals addressed to ascertain both circumstances of death and possible related crimes and the identity of the corpses. Generally speaking, in case of examining up to a few dead bodies in Italy, a complete autopsy is performed, whilst, when several tens or hundreds of corpses are recovered, the lack of resources on one hand and clearer clues on incident, connected crimes, and cause of deaths on the other, push the public prosecutor to limit the request of complete autopsies. In some cases, the dead migrants were identified through visual recognition by relatives, friends, or travel companions. The DVI Interpol protocol is never completely applied to dead migrants for several reasons, mainly for the huge difficulties in retrieving AM data of the missing persons and for some limitations affecting both the primary and the secondary identifiers. The few chances of identification by dental data are further reduced by the systematic lack of an odontologist among the forensic teams charged of the PM; valuable dental data for body identification or for constructing the biological profile of the missing person (age, ancestry, country of provenance/residence, etc.) are likely to be overlooked.
This approach implies a clear disparity with the approach applied when corpses of citizens of the EU or other developed countries are involved and undergo identification. The dead migrants' identification activity should be reconsidered for an improvement in the common international effort in accordance

to an approach more respectful toward the legal rights and dignity of the dead migrants and their families.

Defining the issue

The migratory flows to Europe from the African countries, Asia, and Middle East, have hugely intensified in the recent years to become an important humanitarian issue, and even a great risk of socio-political turmoil for the host countries and for the EU as a whole. The countries in the so called "Mediterranean route", such as Italy and Greece, are, since the beginning, in the front line to cope with the phenomenon, especially nowadays when the so-called "Balkan migratory route" has been interrupted by the contingent policies of countries such as Turkey and Hungary among others.[1,2]

In 2016, more than 98,000 out of a total of 260,000 migrants across the Mediterranean Sea arrived in Italy and in May 2017, the trend of arrivals compared to previous years was: Italy + 576%; Greece – 39%.[3]

The migration flow implied the occurrence of more than 5000 migrants' deaths during the crossing, 1530 in just the first four months of 2017, which are definitely very high numbers. It was able to constitute an important ethical and humanitarian problem and put to the test the whole range of the services provided by the host country's government. 95% of the migrants who arrived in Italy come from African countries, where Nigeria and Eritrea lead the list, being irrelevant to the middle-eastern and Asiatic component.[4]

However, no evidences show that the European continent would really be the desired destination of the migrants' journey. They initially left their home country to follow the job market in the neighbouring countries (mainly Sudan and Egypt) and, at a certain time, were unable to obtain the desired improvement of life conditions, and after a long and often dangerous moving, tried to migrate to Europe. This was done with the help of family networks of friends or relatives already in Europe, who organize and/or fund a part or the full journey. This local migration created specific risks, as smuggling operations developed into trafficking, once migration had begun. There is a clear legal distinction between smuggling and trafficking, but in practice, these processes may become indistinct.[5,6] Smugglers contacted by individuals to facilitate their journey can sell them to traffickers, who extort more money through mistreatment, sexual exploitation or forced labour. From a recent survey on 2387 migrants published by IOM (April 2017), it emerged that migrants who experienced at least one type of exploitation came from Senegal, Ghana, Cameroon, Bangladesh, and Mali (> 93% of positive responses), whilst Pakistanis, Afghans (31%), and Iraqis (9%) had the lowest percentage. Exploitation events were reported to take place mostly in Libya (91% of all cases), followed by Algeria (2%), and other Countries (Greece, Iran, Nigeria ≈1%).[7]

Those migrants who have paid in advance for their entire journey or those who are deemed of being relatively wealthy in their countries of origin, are most at risk. Moreover, some migrants reported that they had left without telling family members in their countries of origin, for fear that they would try to prevent them. This fact creates specific difficulties in retrieving and contacting families for identification purposes when fatalities occur somewhere along the journey. The people on the move leave few or no traces due to the irregularity of their travelling, which can last years, making it actually impossible to identify the Country or the village of origin to search families.

The first and the most important problem that affects rescuing operations of migrants can be identified as the huge difficulties the government faces in funding the missions and in the logistic difficulties of transportation of the migrants recovered at sea. In the very front line of the Mediterranean route, the migrants find Italy, a big and friendly country but since many years of struggling with a financial crisis, a stagnating economy and, above all, continuous political fight about the policy to be adopted to cope with the impressive arrivals of migrants, who will be mostly judged as economic migrants, not refugees. Libya is without a government since 2011, with the result that people who embarked there cannot be sent back, thus, most of them end up staying in Europe despite being denied asylum. Many political voices in Italy say that the EU seems to have left Italy with the responsibility of managing and financing the migrants' reception and ID program on its own, as the relocation program runs very slowly and does not work properly. The chronic shortage of national financial resources, and especially those dedicated to the humanitarian missions, strains the whole system of national reception, from the first phase of the rescue operations at sea to the establishment and management of sanctuaries, and there are huge problems which often cause the ignition of the political debate about sustainability and soon becoming a serious concern for the social peace and the consequent security issues.

Some migrants die before touching the sole of the European continent, during the crossing, often afforded with ships unsuitable for this type of transportation and made available by unscrupulous smugglers or criminal organizations. Afraid of police, the smugglers provide boats with enough fuel just to reach international waters. Migrants are sometime thrown into the sea or abandoned by smugglers, thus, condemned to wait in the water for international rescuing services from Italy or the other nations involved in the patrolling operations.

The tremendous occurrence of migrant casualties during the Mediterranean Sea crossing remains underestimated. We can count only the recovered bodies, so, the real amount of deceased people remains unknown. Therefore, nobody, country officers or databanks[8-10], can provide a reliable number of dead bodies in such a large and now endemic phenomenon. Often the number of the disappeared migrants is calculated by simply putting together pieces of information collected from the shocked and sick survivors. The Missing Migrant Project website and the Migrant Files speak of some thousands fatalities, confirming that the Mediterranean route is most dangerous due to the highest number of deaths in comparison to other routes.

The casualties are usually few, even if sometimes there are large mass disasters with several hundred bodies. Table 1 shows the data of some rescuing operations or disembarks in southern Italy in 2016 and it can be noted that the number of the dead bodies varies from few up to a few dozens.

Table 1: Rescuing operations or disembarks in southern Italy in 2016

SITE OF ARRIVAL	DATE	RESCUED PEOPLE	DEAD BODIES
CATANIA (CT)	16/01/2016	246	1
TARANTO (TA)	30/01/2016	411	6
SICULIANA (AG)	19/02/2016	15	1
AUGUSTA (SR)	24/02/2016	534	5
REGGIO DI CALABRIA (RC)	17/03/2016	591	1
CATANIA (CT)	20/03/2016	254	1
PORTO EMPEDOCLE (AG)	26/05/2016	540	5
REGGIO DI CALABRIA (RC)	29/05/2016	626	45
BRINDISI (BR)	30/05/2016	346	1
AUGUSTA (SR)	04/06/2016	221	1
PORTO EMPEDOCLE (AG)	14/06/2016	242	1
CATANIA (CT)	24/06/2016	745	1
POZZALLO (RG)	30/07/2016	263	1
CATANIA (CT)	02/08/2016	409	4
MESSINA (ME)	02/08/2016	723	1

The recovery of the corpses during the rescuing operation in the open sea poses a specific logistic problem related to the ships which are to be used for the migrants' rescue and transportation procedures, considering also that often the rescuers find the living and the dead bodies at the same time and in the same boat. The dead bodies should be kept in a dedicated refrigerated containers. However, very few of the higher tonnage ships are provided with them. Most ships are provided with only the basic space to separate the corpses from the living. The boats supplied with the refrigerated containers usually take cadavers from other boats not similarly equipped. The temporary storage of the dead bodies in the smaller and not-equipped ships raises logistic problems, which are increased by the high temperatures reached seasonally in the Mediterranean area. However, in some cases, the corpses not immediately recovered or not properly stored appear in a clear state of decomposition[11], thus dramatically reducing the chances of identification, that, as it will be later discussed, in the luckiest circumstances is linked to the possibility of a visual recognition by travel companions or relatives.

In the worst circumstances, the boat sinks with the bodies locked inside. In these fortunately rare occurrences, the recovery of the bodies require firstly, the raising of the boat from the bottom of the sea. The recovery of boats costs millions of euros, as we learned from the Costa Concordia sunk, and sometimes, the governmental offices cannot afford the expenses of such complex and costly operations.

Just as an example, the scale of resources deployed during the recovery of about 700 migrants[12,13], who died in a wooden boat that sunk 137 km from the Libyan coast in April 2015, we can say that three big Italian Navy vessels with submarine robots were deployed to reach the corpses lying at 370 m under the sea level. The corpses were then carried with large transport barges properly equipped with liquid nitrogen refrigeration systems, a complex operation which cost the Italian government about 9.5 million euros. The Italian Government funded the recovery of the boat and the corpses inside it, but no financial support was available for body identification and the dedicated forensic personnel. Many forensic professionals and some postgraduate students in Legal Medicine volunteered to perform the body examinations. The identification of the victims was deemed as highly difficult and with little chances even through DNA.[14] At the moment, 551 corpses had been buried after PM examinations and hopefully, some AM data could be retrieved through international cooperation and at least some unknown bodies might be identified at a later date.[15]

This paper aims to discuss the specific forensic issues connected with identification of the migrants recovered from the Mediterranean sea. The identification procedures of the dead bodies and the examinations addressed to establish the causes of death and possible crime emerge as quite different than the ordinary forensic cases. Some discussion is dedicated to the utility of the primary identifiers, resulting in the conclusion that the ID process is highly affected by the availability and retrievability of ante-mortem data to be compared with PM for migrants' unidentified dead bodies. Specific attention is dedicated to dental data collection that emerged to be underused as primary identifiers, both for being forensic odontologists in DVI teams less involved in the procedures and for the specific features of dead migrants.

The role of the forensic examiners: cause of death, crime investigation, and struggling for a (im)possible identification

Forensic interventions to examine migrants' corpses are ideally required to perform the usual activity and to answer the routine questions about the causes of death by detecting signs of possible crimes and body identification. In practice, several specific issues and limits challenge the activity of forensic professionals addressed to ascertain the circumstances of death, the possible related crimes, and the identity of the dead.

The responsible person of the investigative operations in Italy is in charge of the Public Prosecutor (PP) who is committed to ascertain if any event-related crime has been committed (human trafficking, smuggling, delay of rescuing, etc.), while the identification of the dead bodies is marginally relevant for the PP, especially when no crime was suspected. Most of the times the PP charges for PM examination police forensic officers and forensic pathologists, the latter being called to ascertain the manner and the cause of death. The cause of death is sometimes difficult to be assessed (unknown in 23% according to

Borderdeaths data)[16], whilst in some cases it can be attributed to drowning, sometimes even possibly due to the delay in the rescue operations or choking (sometimes in mass) caused by the overcrowding in the scarce spaces of the ships. The data reported by UNCHR and Borderdeaths reveal the prevalence of drowning (> 50%), followed at a great distance by other causes of death (< 5%), dehydration, suffocation, burnt, violence, etc. Forensics involved in body examination also search for any possible lesions or signs of torture or violence as fractures and/or scars, on the migrant's dead body to determine if any possible crime causally related to torture or slavery at home or any possible traffickers' or smugglers' crimes had been committed. Some forensic pathologists or specialists in legal medicine (who provided medico-legal report to victims of torture) in Italy often faced the physical evidence of different techniques of torture adopted in different areas of the world. Forensic odontologists, when involved in the procedure, could contribute to this crucial process, since head-neck districts are often affected by injuries and some mouth lesions correspond to the torture perpetrated in some specific countries. Through migrants' interviews, we know that some of them had their teeth pulled out or injured in Lybia.[17] Hence, describing properly the crucial evidences found on the body and the mouth, the odontologist may provide relevant information about the origin of the victims or at least the country where they came from and were submitted to torture practices. Last and Spijkerboer et al.[18] highlighted that the aggregation of the data about the cause of death could help in measuring the entity of death occurrences in the Mediterranean crossing and to study the phenomenon at large, but privacy issues and the different coding systems adopted to register the PM data obstruct the large collection and the analysis of these data.

In the first large mass disaster in the Mediterranean Sea that required a DVI response from Italy in October 2013, the death of 366 migrants occurred. The DVI team of Police was activated and police personnel were deployed in Lampedusa island. No civilian forensic experts were recruited due to the lack of funds and organization, as also international observers have later highlighted.[19] The Police forensic officers worked under the most unfavourable conditions due to the large number of cadavers and the lack of refrigerated containers. The teams were forced to examine more than 50 corpses per day with exhausting working sessions and some inexperienced operators reported psychological negative effects. The PM activities consisted of body examination, description of belongings, taking of photos and fingerprints, and biological sampling. Some dental data was scantly registered by the Police forensic pathologist, since no forensic odontologists or dentists were involved and nobody appropriately filled the Interpol 600s forms. Actually, some forensic odontologists, among whom the author is one, were alerted in the first response, but economic and logistic issues lead the authorities to use only the very few Police forensic experts.

This first large disaster depicted the real scenario and the activities that were realistic to provide in such circumstances. In other words, it was the first time that Italy experienced the tremendous issues and limitations concerned with post-mortem and ID procedures in case of irregular migrant deaths. Lampedusa 2013 disaster captured and shocked the public opinion, but dictated a sort of guidelines for body examination that, according to the data collected through personal contacts taken with Police and forensic pathologists involved in Sicily and Calabria, continue to be currently applied.

Generally speaking, in case of examining up to a few dead bodies, a complete autopsy is requested by the PP, whilst, when several tens or hundreds of corpses are recovered, the lack of resources on one hand and clear clues on the incident, connected crimes and cause of deaths on the other, push the PP to limit the request of complete autopsies. Nevertheless, in the latter circumstances some basic procedures are applied to collect at least some PM data, which will be possibly useful for later identification. The bodies undergo an external examination, fingerprints, photos, and biological (bone) sample collection and only in few specific cases, a full autopsy is ordered by the PP. In any case, forensic odontologists are very rarely called to work with the forensic pathologists in large mass disasters, and in cases with few dead bodies.

Even if this approach is quite far from a complete rigorous application of Interpol DVI protocol, the handling of PM exams and identification of migrants in Italy seems slightly better compared to what is generally performed in Greece, where identification issues are rarely addressed by competent authorities. Meaningful inputs are discussed by Kovras and Robins (2016) who described the situation in Greece as follows: "*There is no standardized procedure to deal with a migrant body, and this policy vacuum legitimizes local authorities in denying their legal and moral responsibility to address the issue of identification. Most often relevant data found on the body – documents, tattoos, other identifying marks – are not systematically collected, analyzed and stored to support identification. Similarly, only a limited effort is made to collect other information – such as testimony from survivors of a shipwreck – that could advance this goal*".[19]

In any case, there is a widespread convincement also in Italy that a general description of the corpse and the sampling of bone as a source of DNA is more than enough for dead migrants; a wider effort tends to be considered useless also by forensic experts, that experienced frustration for the lack of interests by national and international authorities especially in retrieving AM data of missing migrants. There are no adequate response and deployment of resources and funds, thus, ending to feel that most of their efforts result ultimately useless and identification of dead migrants is a poorly motivated and then, an impossible mission.

The identification of the dead bodies should be best performed according to the usual Interpol protocol, which enables the only true scientific ascertainment of identity by the deployment of DVI teams properly composed of all the needed experts to collect and examine primary and secondary identifiers. In some cases, the dead migrants were identified through visual recognition by relatives, friends, or travel companions, but we know that visual recognition can be biased by several factors and cannot be considered a proper way to identify a corpse.

The DVI Interpol protocol is never completely applied for dead migrants for several reasons, mainly due to the huge difficulties in retrieving AM data of missing persons, some limitations affecting both primary and secondary identifiers and some not adequate response and operational organization.[20,21] Fingerprints are useless after the corpse has been in the water for hours or days and even in the cases in which fingerprints are obtained, they become useless since the countries which the migrants mostly come from do not have connections with any international fingerprint data banks to enable the necessary comparisons for identification. The Commission report to the EU Parliament and Council in 2016 on SIS II reported the impressive number of fingerprints retained in the AFIS databank and the number of fingerprint identification performed in the Schengen area per day.[22] Nonetheless, most of the countries from which the immigrants started their journey , do not adhere to international programs of identification of travelers/visa or document check and have few or no connections with international fingerprints databanks. Fingerprints resulted, registered at their arrival in Italian hotspots (in Pozzallo, Porto Empedocle, e.g. in Sicily), are indeed decisive for identifying some irregular migrants who died after moving across Italy.[23-25]

The dental data do not help as many of the migrants were young and had very few dental treatments done to be possibly used for matching with AM dental data that might be possibly searched in the country of origin because, differently from fingerprints, no registration of dental data is made to migrants at their arrival, neither in case of dental cares received in Italy. When dental treatments are detected in unknown corpses, the chance to identify the dental practitioner and collect the dental file of the missing patient seems very unlikely. A list of the passengers of the boats is obviously unavailable, and it is not possible to obtain AM data to compare, since the migrant's country of origin is not known as well as any possible personal dentist's name. Sometimes the dental features can be compared with AM photos or at least recognized by families, but these evidences are far from being scientifically acceptable.

The few chances of identification by dental data are further reduced by the systematic lack of an odontologist among the forensic teams in charge of the PM examinations. Generally, only a forensic pathologist is called in Italy to provide the external examination or autopsy and police officers intervene in order to take fingerprints and photos, thus, concretely risking to overlook the collection of valuable dental data for body identification or for constructing the biological profile of the missing person (age, ancestry, country of origin/residence, etc.).

Therefore, the DNA collection offers the only option to try an identification of the dead body. However, many problems arise also in this case: most of the time, the lack of information about data such as country of origin, the name of the body, and its family completely jeopardizes the possibility of matching the data retrieved from the DNA analysis. Moreover, there is not a clear shared opinion about which national or foreign governmental institution should contact families and afford the costs of the whole ID procedure. However, very often, such piece of information is not available from friends and trip mates since many of the migrants usually lie about their home country to avoid any possibility of police/ government retaliation against their families at home who often live in difficult socio-political conditions or in war-ridden countries.

Given the many limitations that affect ID procedures, largely due to poor efforts in retrieving AM data and to the incomplete application of fair and scientifically accredited PM approaches, with no PM examinations, anybody can be surprised by the low rate of positive identifications of migrants, especially if coming from Africa. According to the "Deaths at the Borders" database, two thirds of the migrants who arrived in southern Europe from 1990 to 2013 were not identified and people coming from sub-Saharan Africa remain more likely to be unidentified compared to Asians. Moreover, children or people older than 40 are more likely to be identified since they usually travel with relatives or friends, but many people illegally crossing the Mediterranean Sea are between 20 and 40, and the corpses have less chances to be identified.[26]

CONCLUSION

The Mediterranean Sea route is the most dangerous and the increase in the immigration flows goes well with the rise in deaths and corpses to be identified.

Very few corpses are identified so far with a family and trip mates' visual identification procedure, therefore, implying that the chances of positive ID are bound to the contingency that the dead individual crossed the sea with other people who survived.

The ID procedures for migrants in Italy are limited to the collection of some very essential data because very rarely it is possible to wholly apply the Interpol DVI protocol[27] with the proper deployment of all the necessary professionals. The PM examination is generally not extended to consider all the primary and secondary identifiers and is performed by teams in which the required participation of some essential professionals according the Interpol DVI guidelines, such the odontologist, is quite systematically lacking.

Dental data of dead migrants, even if unlikely useful for a comparison with hardly obtainable AM data, are likely to be largely overlooked either for the identification or for the description of the biological profile of the corpse. Some essential information about the country where migrants experienced tortures featuring mouth lesions are likely to be neglected.

This approach implies a clear disparity of approach compared to situations in which corpses of citizens of EU or other developed countries are involved and undergo identification. The dead migrants' ID activity should be reconsidered for an improvement in a common international effort according to an approach more respectful of the legal rights and the dignity of dead migrants and their families. Italy, on the other hand, is facing the immigration emergency with a scarcity of financial resources, moving from a general condition in which the Interpol protocol is hardly applied completely and uniformly in any case of an unidentified dead body. However, the real issue, seems to be not the lack of financial resources, a problem which might be considered as a valid issue only in those very costly cases of boat recovery. The lack of a valid organization is deemed the main issue, without a careful and clever optimization of the financial and professional resources, indeed largely available in Italy, and in the international scarce interest in the ID of the migrants, since, once verified, their condition of migrants and the awareness that identification has few chances of success, only some basic investigations are granted. On the contrary, identification of a human being should be strongly sought in order to restore dignity and to give relief to the families left behind. Moreover, the ID of a body enables the protection of the many legal rights (marriage, inheritance, etc.) of the relatives involved. The effort in persuading the local authorities of the importance of the ID procedures must certainly be a political one, providing the necessary support to the national or international DVI teams. The identification procedures should be standardized and refined with a common effort, to the best application of the DVI Interpol protocol, firstly involving all the experts in treating primary identifiers data, and especially forensic odontologists that, at the moment, are seldom involved, and secondly, enhancing the improvement of the international databank of unknown dead bodies. However, we are all aware that the greatest weakness of the identification procedure lies in the AM data collection, and the cooperation of the national and International Committee of the Red Cross and the NGOs, whose networks could help in retrieving families and contacting them without any threat for an unfavourable political context, could be of great importance.

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