A systematic review of odontological sex estimation methods

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The authors declare that they have no conflict of interest.

KEYWORDS

sex estimation,
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ABSTRACT

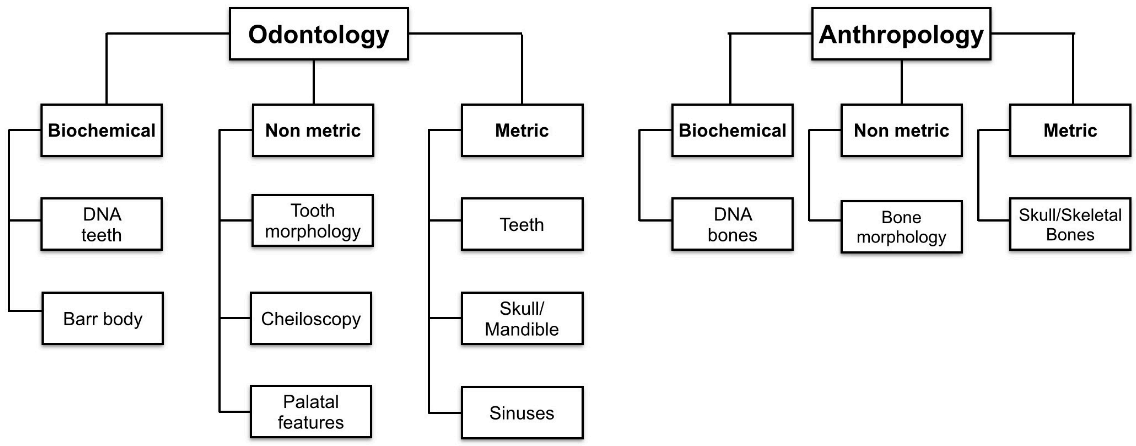
Background: In human identification sex estimation plays an important role in the search for ante-mortem data.
Aim: To systematically review studies describing and testing/validating methods of odontological sex estimation. The set research question was: What odontological sex estimation method is the 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

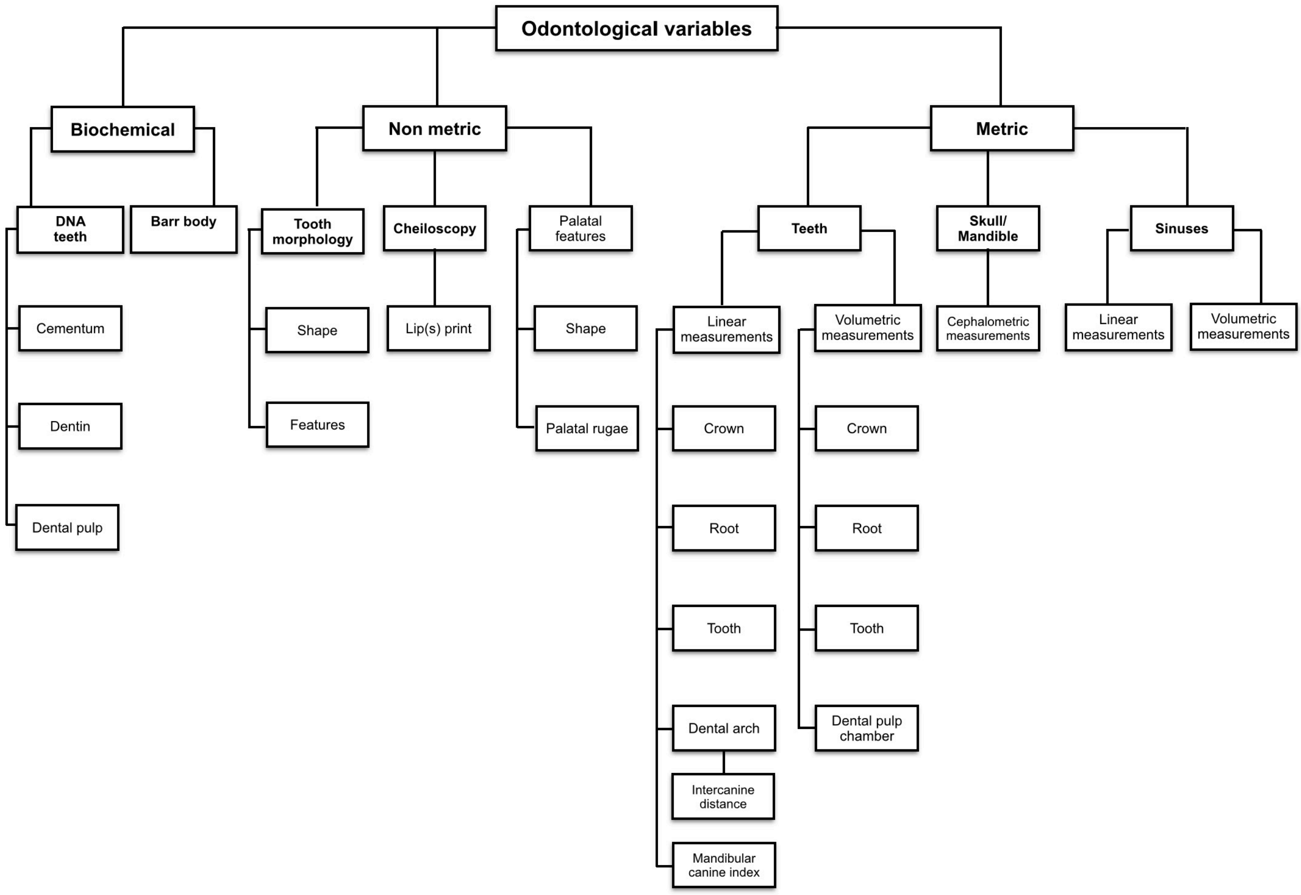
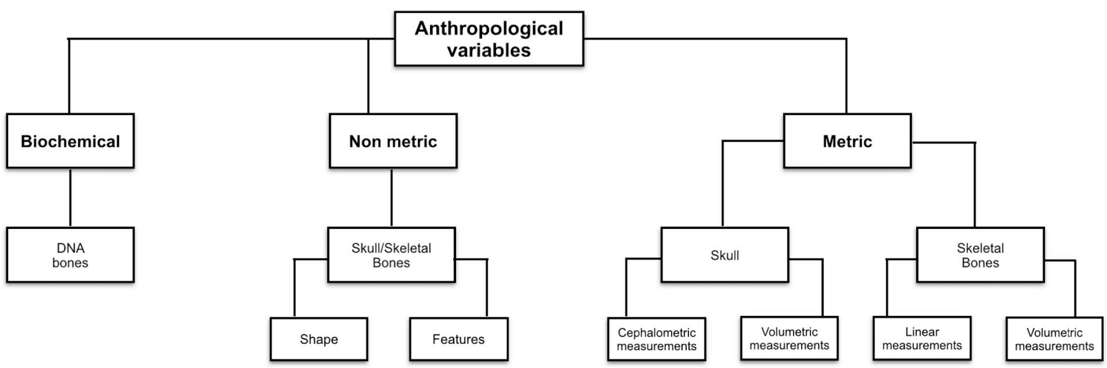


Fig.3: Flow chart of possible parameters for anthropological sex estimation



MATERIALS AND METHODS:

An electronic search until November 29^{th} 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)

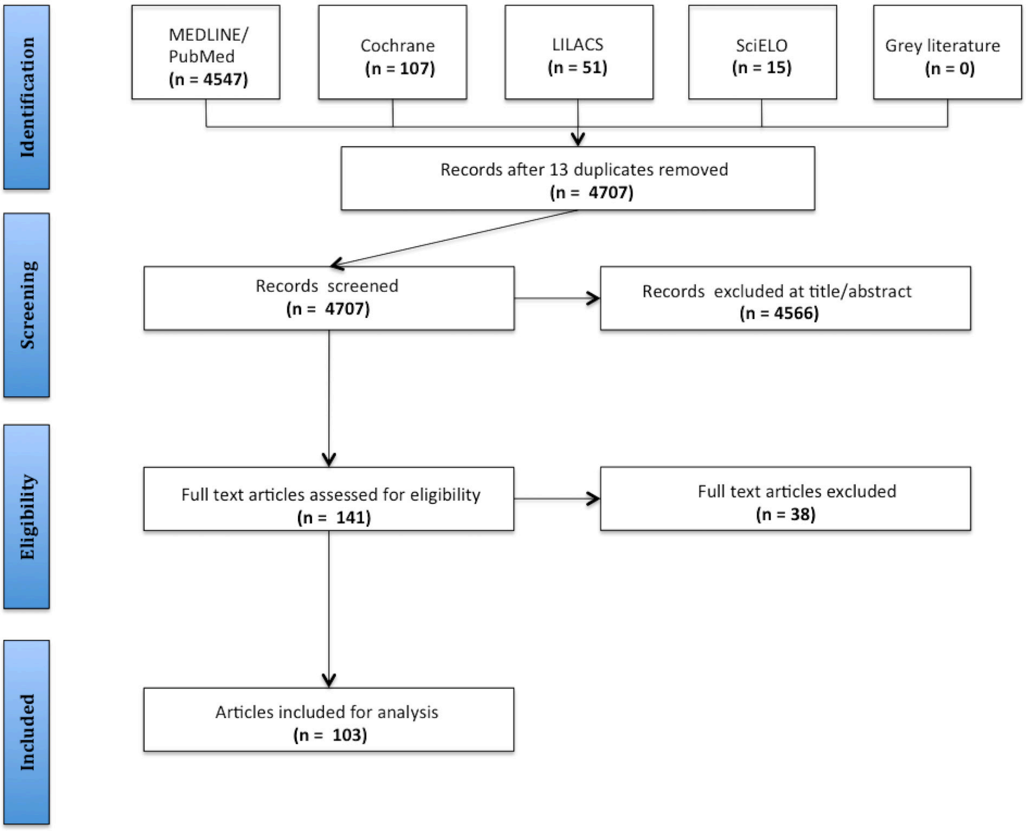
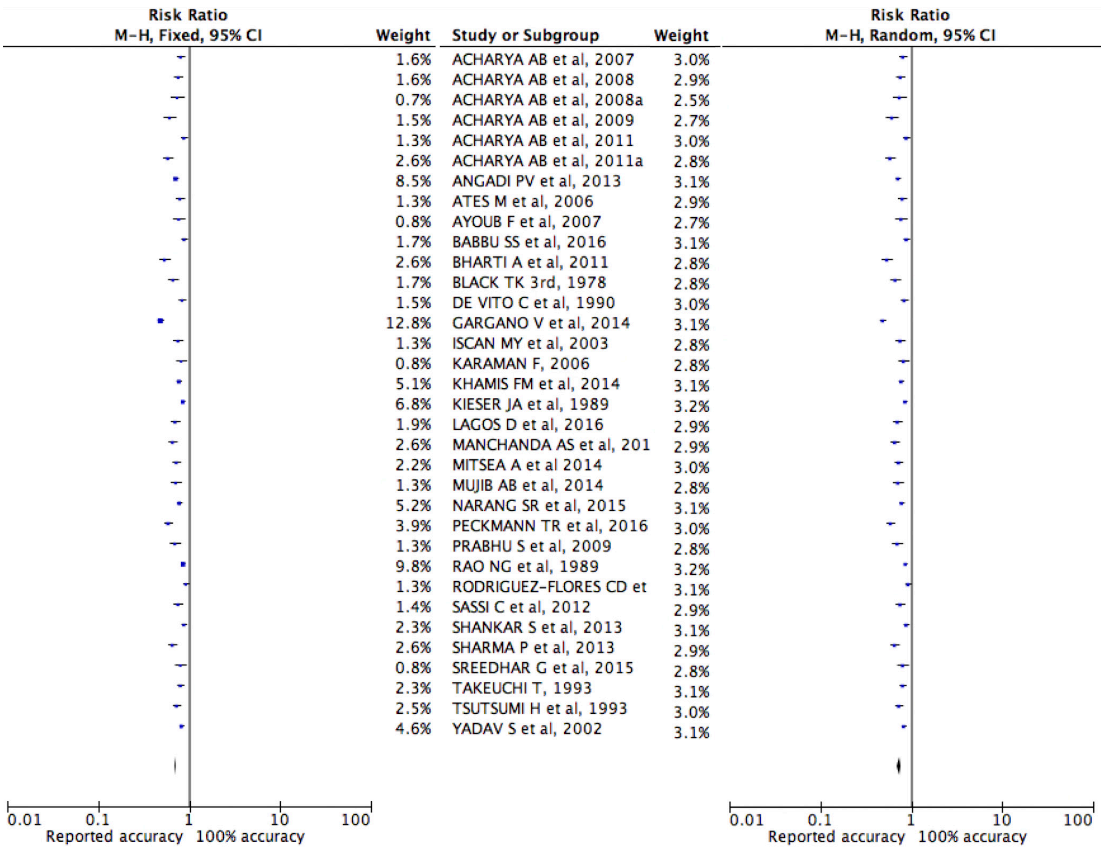


Fig.5: Meta-analysis on the accuracy of sex estimation studies based on odontometric measurements on casts. (M-H = Mantel–Haenszel test, CI = confidence interval)



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
Biochemical	KOMURO et al, 1998	20 (10M/10F)				100	4
Biochemical	URBANI C et al, 1999	94 (50M/44F)				100	6
Biochemical	MURAKAMI H et al, 2000	129 (64M/65F)				83.33-100	4
Biochemical	SIVAGAMI AV et al, 2000	24	10-85	18,17,11,23,24,31,33,34,46	Indian	100	5
Biochemical	VEERARAGHAVAN G et al, 2010	60 (30M/30F)	18-74	maxillary and mandibular PM/M	Indian	65-100	7
Biochemical	SUAZO GI et al, 2010	40 (20M, 20F)	24-45	PM/M		100	5
Biochemical	SUAZO GI et al, 2011	50 (25M/25F)	14-44		Chilean	100	7
Biochemical	ZAPICO SC et al, 2013	14				100	5
Biochemical	KHORATE MM et al, 2014	100 (50M/50F)				100	6
Biochemical	ZAGGA AD et al, 2014	9		deciduous (I)/ permanent teeth(C,PM,M)	Nigerian Black/White	100	6
Biochemical	SANDOVAL C et al, 2014	56 (28M/28F)	15-45	PM/M3		98.9	5
Biochemical	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
Cheilioscopy	SHARMA V et al, 2014	200 (100M/100F)	17-26	Lips	Indian	81	7
Cheilioscopy	NAGALAXMI V et al, 2014	60 (30M/30F)	20-30	Lips	Indian	85.05	7
Cheilioscopy	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
Palatal Features	SARAF A et al, 2011	120 (60M/60F)	22-26	Palatal Rugae	Indian	99.15	8
Palatal Features	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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