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Dental techniques for sexual diagnosis in forensic medicine: an Integrative systematic review

Marie-Lou Petit dit Dariel

Dissertação conducente ao Grau de Mestre em

Medicina Dentária (Ciclo Integrado)

Gandra, 25 de julho de 2020

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Trabalho realizado sob a Orientação de Alexandra Teixeira

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To my mother, who has always supported me. My admiration is limitless and I wouldn't be who I am today without you. I learned everything from you and even more. Thank you for everything.

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ABSTRACT

Human identification by dental means is the subject of forensic odontology. In the absence of antemortem information or when human remains are highly fragmented or degraded, for Instance in case of explosive blasts or natural disasters, teeth, due to their robust nature, are an excellent study material. During criminal or mass disaster investigations, determining a person's sex becomes the priority in the process of identification by a forensic investigator, since it decreases the number of potential candidates by half. Therefore, sex estimation by dental methods is an important parameter in the identification process.

The objective of this dissertation is to review the various dental methods that can be applied in sex evaluation in a forensic context. In this work, amelogenin was the most utilized gene for sex determination analysis, following DNA extraction and amplification by PCR (Polymerase Chain Reaction). This methodology shows exceptionally reliable results, with 75%-100% correct identification of the samples analyzed in the studies reviewed in the present work. When using morphologic methods, the mandibular canine is the tooth most frequently analyzed (6/13 articles) and that shows the most sexual dimorphism (72,5-92.3% accuracy). For this reason, the mandibular canine index (85% accuracy in an Indian population) is a method frequently used, however, it is population dependent. Alternative methods for sex evaluation are therefore an interesting area of research and amongst these, the use of CT scans to evaluate mandibles are expected to be further developed and used in the future.

KEYWORDS

Biological profile; DNA molecular analysis; Forensic dentistry; Human Identification; odontometry, Sex determination.

INDEX

1) INTRODUCTION

The application of dental standards to legal problems is known as forensic odontology. One particularly important role of the forensic odontologist is to contribute to Human identification, which can be achieved using dental records. (1). This constitutes a primary method of identification (together with fingerprints and DNA typing). These are comparative methods which means that antemortem data must be available. Moreover, dental recognition may be challenging when there is a long temporal gap between antemortem and postmortem records. (2)

In the absence of antemortem information or in circumstances where visual recognition is impossible and/or human remains are highly fragmented or degraded, teeth, due to their robust nature, are an excellent study material (3,4). In this case, reconstructive methods are used, which evaluate parameters of the biological profile (sex, stature, ancestry, and age) that can provide relevant information about the individual (3). Amongst these, sex determination is crucial, since it cuts by half the number of possible individuals to whom the remains belong.

There are several dental methods for sex estimation, which can be divided into morphological and molecular. Morphological methods can be performed on hard tissues to evaluate teeth morphology and dimensions (orthometric; odontometric methods). These methods can also be applied to soft tissues (lip prints-Cheiloscopy, palatal rugae pattern-Rugoscopy), although these are limited studies, sometimes with contradictory results (5).

Molecular methods include DNA analysis of the sex chromosomes and/or the amelogenin gene and cytology to evaluate the presence of Barr bodies in females (6). Since DNA can be extracted from teeth long after the individual's death and morphological patterns may be affected by external factors, molecular analysis of DNA is a good method for determining sex. (7) However, in a forensic context, several factors such as the number of victims to identify, level of integrity of the human remains, exposure to extreme conditions (such as fire or water), the place where the remains were found and local technical capacity to perform molecular biology analysis, must be

taken into consideration, to select the best method to be applied in each particular case. (1)

2) OBJECTIVES

The objective of this work is to review the most well-known dental methods and techniques that can be used to evaluate sexual diagnosis in a forensic context.

3) MATERIALS AND METHODS

Within the scope of the theme, this dissertation was carried out using a bibliographic search in the PubMed database. Full text articles concerning research in humans, written in English; French and Portuguese were searched, using the combinations with the following terms:

“Sex determination AND dentistry AND DNA” OR “DNA AND forensic dentistry” OR “sex determination AND forensic dentistry” OR “human identification AND forensic dentistry”.

A total number of 225 articles was obtained in the PubMed database search and 3 articles through other sources.

The total number of articles was compiled for each combination of key terms and therefore duplicates were removed using Mendeley's quote manager. A preliminary evaluation of the abstracts was carried out to determine whether the articles met the objective of the study. The selected articles were read and evaluated individually as to the purpose of this study.

A total of 23 articles were considered relevant to the theme of the work, corresponding to research work published In English, between 2008 and 2019 (See figure 1- Prisma flow diagram).

To contextualize the subject theme, a total number 3 book publications and 12 reviews/meta-analysis were also consulted and included in the bibliography, that comprises a total of 38 publications.

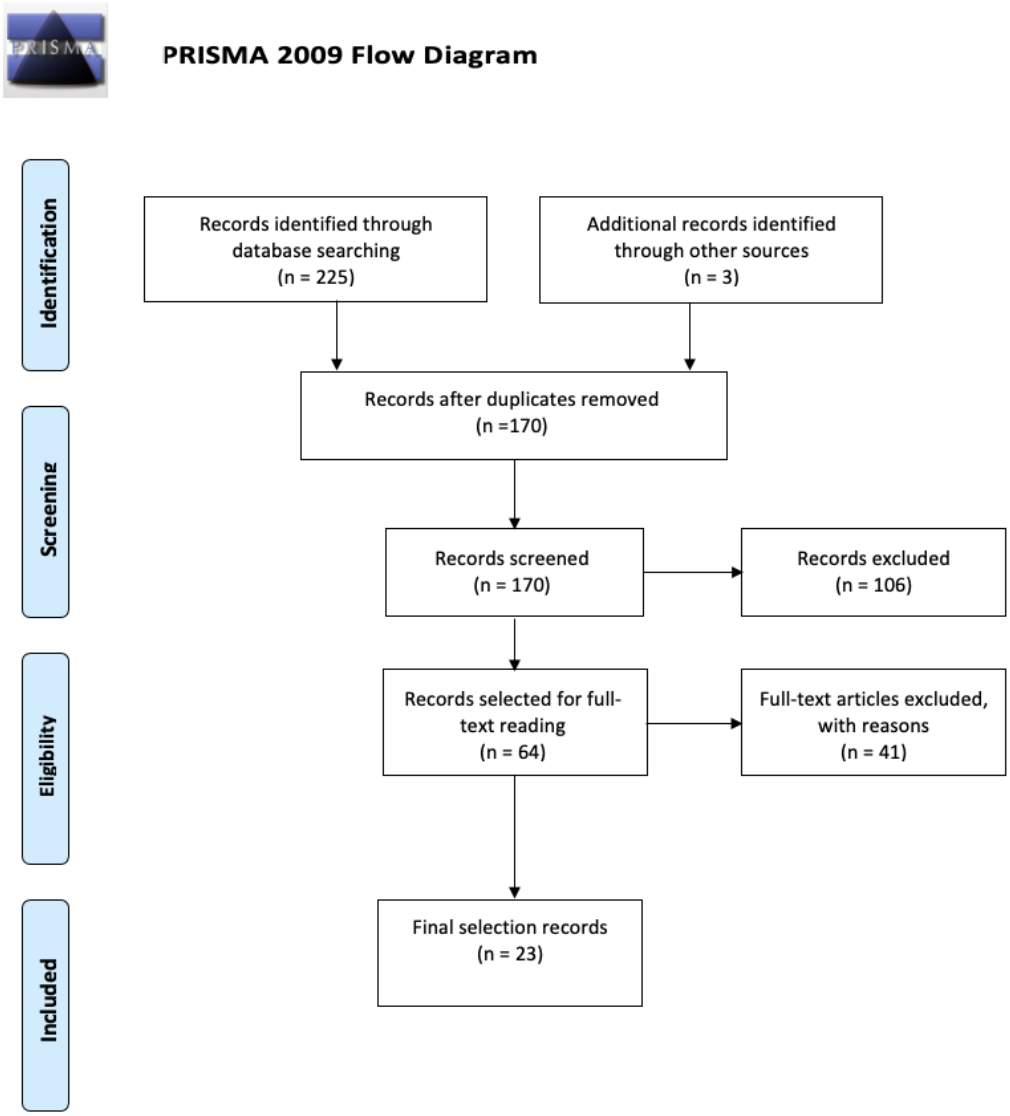

Figure 1 - Prisma flow diagram showing the different stages of article selection

4) RESULTS

Table 1. Relevant data extracted from the selected articles.

References and links	Objectives	Methods	Results
MOLECULAR METHODS			
Sex determination by amplification of amelogenin gene from dental pulp tissue by polymerase chain reaction. Chowdhury RM et al., 2018 (2)	sex evaluation by amplification of the amelogenin gene by polymerase chain reaction (PCR) using DNA extracted from dental pulp, which was exposed to various environmental conditions created artificially to mimic a forensic scenario.	A total of 130 extracted premolars were subjected to various conditions imitating a forensic scene. A) desiccated at room temperature for a period of 30–90 days; B) submerged in salt water for a period between 30 days and 90 days; C) buried in soil for a period between 30 days and 90 days; D) subjected to high temperatures between 150°C and 350°C. DNA was extracted from dental pulp tissue and sex determination was achieved by amplification of the amelogenin gene by PCR.	DNA could be extracted from all samples except from those that were subjected to a temperature of 350 °c. DNA amplification and sex determination of the samples were found to be accurate
Sex determination from mesiodens of Indian children by amelogenin gene. Srivastava M et al., 2018 (7)	To determine sex from mesiodens, following DNA extraction and amelogenin analysis by polymerase chain reaction (PCR).	DNA was Isolated from eight human-extracted mesiodens and was subjected to PCR analysis using predesigned primers for amelogenin (genes. AMEL) x and AMEL y	DNA could not be obtained in two samples. Sex determination was successful in six out of eight samples (75%)
Sex determination of human remains from peptides in tooth enamel. Stewart NA, et al., 2017 (8)	Correctly assign sex to archaeological human remains of various chronological ages, from hundreds to thousands of years old.	A minimally destructive surface acid etching of tooth enamel and subsequent identification of sex chromosome-linked isoforms of amelogenin, an enamel-forming protein, by nanoflow liquid chromatography mass spectrometry.	sex determination in archaeological human remains of various chronological ages, from hundreds to thousands of years old was effective.

A new method for sex determination based on detection of SRY, STS and amelogenin gene regions with simultaneous amplification of their homologous sequences by a multiplex PCR. Morikawaa T er al., 2011 [11]	To determine sex based on detection of SRY, STS and Amelogenin gene regions with Simultaneous Amplification of Their Homologous Sequences	Multiplex PCR: Simultaneous detection of the SRY (sex-determining region Y), STS (steroid sulfatase) and amelogenin (AMELX and AMELY) gene regions and their homologous sequences.	Sex determination using this method was accurate and reliable. It may be used to correctly identify the sex of males presenting and AMELY-deleted gene. Also useful in the sexual evaluation of forensic autopsy samples where DNA degradation might have occurred due to environmental exposure.
Rapid and simple sex determination method from dental pulp by loop-mediated isothermal amplification. Nogami H et al., 2008 (12)	Evaluate sex determination using LAMP method with the amelogenin locus as the index using dental pulp samples, with the object of personal identification of skeletal remains or seriously decayed bodies.	Loop-mediated isothermal amplification (LAMP) method.	The X allele was detected in approximately 32 min with real-time turbidimeter, and the Y allele was detected in approximately 34 min. Analysis time was reduced to half when using loop primers.
Efficacy of Sex Determination from Human Dental Pulp Tissue and its Reliability as a Tool in Forensic Dentistry. Khanna KS.2015 (14)	To define human dental pulp as an important tool for sex evaluation in forensic odontology. To study the time period in which sex can be determined from pulp tissue, using histological methods for Barr body identification.	90 healthy pre-molars and molars in adult patients (45 males and 45 females) were divided into three groups and stored at normal room temperature for 15 days, 1 month, and 2 months. The pulp was obtained through normal access preparations; Barr bodies were analyzed in pulp tissue using three stains: H & E, Feulgen, and acridine - orange.	The female sample cells taken from dental pulp tissue were stained positive for Barr/chromatin bodies. The three different stains used were effective. Feulgen is a better stain then H and E in relation to the qualitative assessment of Barr bodies, which can be detected in pulp from teeth, kept at room temperature up to 2 months. In male samples, cells stained negative or <3% for Barr/chromatin bodies.
Sex determination from dentin and pulp in a medicolegal context. Zapico, SC. & Ubelaker, DH. 2013 (25)	To analyze the accuracy of sex identification from dentin and pulp via DNA isolation and PCR analysis of the amelogenin gene.	DNA was extracted from the dentin and pulp of 14 teeth by using a silica-based methodology. They used the amelogenin gene to determine the sex via PCR. B-actin, a housekeeping gene, was used as a control gene. The authors checked the results in agarose gel and semi quantified them by using gel analysis software.	It was possible to determine a deceased person's sex by analyzing amelogenin gene by PCR, using DNA extracted from the dental pulp and from dentin.
Differential nuclear and mitochondrial DNA preservation in post-mortem teeth with implications for forensic and ancient DNA studies. Higgins D. et al., 2015 (27)	To investigate the DNA distribution and rates of DNA degradation in the different dental tissues over short to medium post-mortem intervals.	Quantitative real time PCR (QPCR) to measure the relative degradation rates across teeth tissues and between nuclear and mitochondrial DNA.	Nuclear and mitochondrial DNA is not distributed evenly throughout teeth and decays at different rates in different tissues, influenced by antemortem and postmortem factors. DNA degradation in teeth is dependent on post-mortem interval and soil

			temperature. Cementum is particularly important for recovery of nuclear DNA as its structural integrity is maintained over extended periods.
The tooth for molecular analysis and identification: a forensic approach. Corte-Real, A et al., 2012 (28)	To optimize laboratory preparation of teeth for DNA identification. Study of the apical radicular portion and the remaining radicular portion to analyze whether they differed in DNA quantity and quality.	Teeth were exposed to different experimental conditions: A) buried in different mediums: pH5-pH7; B) buried in sand for a period of 7 months; C) Exposed to the atmospheric conditions of the Iberian Peninsula, for two years. Total DNA quantification was performed by real time PCR, by using the Human Quantifiler kit (Applied Biosystems®).	The tooth's apical portion is the preferential choice in sample preparation of dental mineralized tissue for molecular analysis and identification.
Sex determination from tooth pulp deoxyribonucleic acid using polymerase chain reaction. Pawar RK & More CB. 2018 (35)	To determine sex from tooth pulp tissues by DNA analysis using polymerase chain reaction amplification of amelogenin, under different environmental conditions.	The human extracted teeth were exposed to different conditions such as heat, soil, and open environment. The DNA was extracted from these teeth, quantified, and further amplified using male (AMELY) and female (AMELX) primers	The accuracy in determining sex from pulp DNA ranged from 92% to 100% in the study groups, except from the teeth exposed to uncontrolled heat, as the pulp tissue was burnt completely
MORPHOLOGIC METHODS			
Contribution of dental tissues to sex determination in modern human populations. García-Campos C, et al., 2018 (9)	To determine the degree of sexual dimorphism in the dental tissue volumes and surface areas of mandibular canines and to explore its potential for reliable sex determination.	The teeth included in this study were selected from anthropological collections. The sex was known. The teeth were scanned and three-dimensional (3D) measurements (volumes and surfaces areas) were obtained. A discriminant function analysis was applied.	Sexual dimorphism in canine size is due to males having greater amounts of dentine, whereas enamel volume does not contribute significantly to overall tooth size dimorphism.
Discriminant canine index - a novel approach in sex determination. Kiran CS, et al., 2015 (15)	The aim is to evaluate the reliability of sex determination using discriminant canine index (DCI)	A total of 120 subjects, with healthy periodontium and between the age groups of 15 to 40 years were selected randomly. Subjects with hard tissue abnormalities were excluded from the study. The maximum mesiodistal widths of left mandibular canines were measured intraorally with the help of divider and digital vernier caliper. Data was collected and analyzed statistically.	A significant increase in the mesiodistal width of canine in males when compared to females was observed. The discriminant canine index (DCI) has an accuracy rate of 72.5%.

Mandibular canine index: a reliable predictor for gender identification using study cast in indian population. Singh. et al., 2015 (16)	To assess the reliability of MCI in sex determination in Indian population.	Dental casts were prepared for all individuals and the measurements of mandibular canine teeth were taken. The MCI was calculated using the standardized equation. In addition, the percentage of sexual dimorphism was calculated.	Sexual dimorphism is more on left permanent mandibular canine teeth than right permanent mandibular canine teeth.
Sex discrimination potential of permanent maxillary molar cusp diameters. Macaluso, P. J. 2010 (17)	To assess the potential usefulness of permanent maxillary molar cusp diameters for sex discrimination of poorly preserved skeletal remains	Cusp diameters were measured from standardized occlusal view photographs. Univariate and multivariate discriminant function equations were performed.	All cusp dimensions for both first and second maxillary molars exhibited significant sexual dimorphism. Discriminant function equations permitted low to moderate classification accuracy in discriminating sex.
Anthropometric analysis of mandible: an important step for sex determination. Alias IA et al., 2018 (20)	Sex evaluation using human mandibles, through morphology, morphometric measurements and discriminant function analysis from CT scans.	Three morphologic and nine morphometric parameters were measured, using Osirix MD Software 3D Volume Rendering.	All parameter measurements showed significantly greater values in males than in females, with an overall accuracy of 78.5%
Sex determination using cheiloscopy and mandibular canine index as a tool in forensic dentistry. Singh J, et al., 2012 (21)	Evaluate the reliability of cheiloscopy and mandibular canine index (MCI) in the determination of sex in an individual, to analyze different lip patterns reproduced by the natural dye and lysochrome dyes and to compare the MCI in males and females for the determination of sex and to check the reliability of cheiloscopy and MCI for the same.	Lip prints were developed using natural dye (vermilion) and lysochrome (Sudan Black II) dyes and their patterns categorized according to Suzuki &Tsuchihashi's classification. MCI were calculated. Analysis of the two was performed.	The results of the present study revealed MCI to be more reliable in the determination of sex than cheiloscopy.
Study of the effect of age changes on lip print pattern and its reliability in sex determination. Randhawa, K. et al., 2011 (22)	To determine the most common lip patterns in North Indian population and evaluate Its usefulness for sex determination.	Cheiloscopy analysis was performed on 600 participants, divided into 3 groups, according to age: Group 1 (1-20 years) - 150 subjects; 72 females and 78 males Group 2 (21-40 years) - 300 subjects; 159 females and 141 males Group 3 (> 41 years) - 150 subjects; 80 females and 70 males.	Statistical analysis showed very highly significant difference for different lip patterns in males and females in group 2 and no significant difference in group 1 and group 3.
Rugae patterns as an adjunct to sex differentiation in forensic identification.	The aim of this study was to identify and compare the rugae patterns in Indian males and females, as an additional method of	Dental casts were obtained from 120 subjects (60 males and 60 females) of Indian origin, aged 22-26 years. Palatal rugae were recorded and	The rugae pattern may be an additional method of differentiation between the Indian male and female.

Saraf A, et al., 2011 (23)	differentiating the sexes in various postmortem scenarios.	classified according to Thomas & Kotze and Kapali.	
Are dental indexes useful in sex assessment? Acharya AB et al., 2008 (29)	To evaluate the usefulness of dental mesiodistal (MD)and buccolingual (BL) indexes for sex evaluation.	123 dental casts from young Nepalese adults (58 females and 65 males) aged 19–28 years were collected. MD and BL measurements of all teeth (excluding third molars) were obtained using a digital calliper and dental indexes were calculated. Sex differences in the dental indexes were assessed using univariate and multivariate statistics and compared to that of linear measurements reported previously on the same sample.	For crown area canines showed the greatest sex dimorphism followed by the maxillary first molar, maxillary central incisor and mandibular second molar.
A novel computer-assisted method of bite mark analysis for gender determination. Maji A. et al., 2018 (30)	To evaluate the bite marks of males and females using a novel indirect computer-assisted method and explicate its application in forensic odontology	Intercanine distance (ICD; a perpendicular line drawn from the midpoint of the upper central incisors to the intercanine line (line AB), and two lines (X and Y) drawn from both the distal aspect of the central incisors to the midpoint of line AB forming angles ABX and angle ABY, were measured. The Kruskal–Wallis test was performed to compare the bite marks of males and females.	The mean ICD of males and females was found to be 32.96 mm and 29.84 mm, respectively, with a mean difference of 3.11 mm and statistically significant p value <0.001. Analysis of bite marks using this novel computer-assisted method may constitute a simple, reliable, easily reproducible, and economical technique. However, further studies, with bigger samples, are needed to validate this technique.
Validation of a physical anthropology methodology using mandibles for gender estimation in a Brazilian population. Carvalho SP et al.,2013 (32)	This study aimed to estimate the sex of skeletons by application of mandible measurements, a method previously used in a population sample from Northeast Brazil.	The method used two mandibular measurements, namely the bigonial distance and the mandibular ramus height. The sample was composed of 66 skulls from the city of Garulhos, in Southeast Brazil. The method was applied by two independent examiners.	In this study, direct application of the previously reported method, achieved a rate of accuracy of 100% for females but only 11% for males (against the reported 81.11% for females and 76.47% for males in the population of Salvador) and may be explained by the ethnic differences between these populations. Statistical adjustment of measurement data for the population analyzed allowed accuracy of 76.47% for males and 78.13% for females, with the creation of a new discriminant formula.
Sex determination by mandibular ramus: A digital orthopantomographic study.	To determine the usefulness of mandibular ramus as well as the anteroposterior |	A retrospective study was conducted using orthopantomographs. Mandibular ramus measurements were carried out using Master	Mandibular ramus measurements can be a useful tool for sex determination.

Samatha K. et al., 2016 (36)	superioinferior angle of mandibular condyle, as an aid in sex determination.	View 3.0 software and subjected to Discriminant function analysis.	
Modern humans sex estimation through dental tissue patterns of maxillary canines. García-Campos C et al.,.2018 (37)	Evaluate dental tissue volumes and surface areas of maxillary permanent canines in a sample of known sex to provide new data and to explore the potential of these variables as reliable sexual estimators.	The teeth were scanned and three-dimensional (3D) measurements (volumes and surface areas) were obtained. In addition, a discriminant function analysis was applied.	Male maxillary canines have a greater dentine component, whereas female enamel is thicker, leading to a difference in dental size in favor of males. 92.3% correctly assigned when all functions were applied together.

There are 10 articles out of 23 that study the various methods for sex evaluation based on molecular traits related to amelogenin gene or peptides, Barr bodies or SRY gene analysis, corresponding to 43,5% of the articles analyzed in this study (Table 2).

Table 2 - Detailed information on selected molecular studies regarding the number of Individuals, age, population origin, sample type, methods used and Sex estimation accuracy.

Study	Included subjects Total, M/F	Age range (years)	Samples Examined	Methodology	Type of study	Population Origin	Sex estimation accuracy Mean (%)
(2) Chowdhury RM et al., 2018	130	18-56	Premolars	In-vitro study: analysis of the amelogenin gene using polymerase chain reaction (PCR) method on DNA isolated from dental pulp	Molecular analysis	India	n.d.
(7) Srivastava M et al., 2018	8	n.d	Mesiodens (supernumerary Teeth)	Amelogenin analysis by polymerase chain reaction (PCR).	Molecular analysis	India	75%
(8) Stewart NA, et al., 2017	21	26-76	Teeth	Nanoflow liquid chromatography mass spectrometry	Molecular analysis	North Yorkshire, United Kingdom. Three archaeological sites ranging in date from the early neolithic (ca. 5,700 BP) to the medieval period	Maximum accuracy of ~ 80–95% Depending on factors as skeletal preservation and degree of sexual

							dimorphism within the sample
(25) Zapico, SC. & Ubelaker, DH. 2013	14 M5/F9	39-70	2 incisors 12 molars	Amelogenin analysis by polymerase chain reaction (PCR).	Molecular analysis	Spain	n.d.
(11) Morikawaa T et al., 2011	246 M204/F42 + 35 forensic cases M27/F8	n.d. n.d pm interval: 2d-5yrs	Blood sample muscle, organs, cartilage, teeth, and bone	Multiplex PCR: Simultaneous detection of the SRY (sex-determining region Y), STS (steroid sulfatase) and amelogenin (AMELX and AMELY) gene regions	Molecular analysis	USA & Japan	100%
(12) Nogami H et al., 2008	32 M21/F11	n.d	Permanent teeth	Loop-mediated isothermal amplification (LAMP) method. Using DNA extracted from dental pulp samples stored at room temperature for at least 25 years after their removal,	Molecular analysis	Japan	100%
(27) Higgins D. et al., 2015	85 M38/F47	16-60	150 third molars	DNA was extracted from coronal dentine, root dentine, cementum, and pulp of 114 teeth via a silica column method and the remaining 36 teeth were examined histologically.	Molecular analysis	Australia	n.d
(28) Corte-Real, A et al., 2012	25	18-87	Teeth	Experimental conditions, Total DNA quantification was performed by real time PCR, by using the Human Quantifiler kit (Applied Biosystems®).	Molecular analysis	Portugal	n.d
(35) Pawar RK & More CB., 2018	200	21-84	Teeth Pulp	Amelogenin analysis by polymerase chain reaction (PCR).	Molecular analysis	India	92% - 100%
(14) Khanna KS., 2015	90 M45/F45	n.d	Pre-molars Molars	Barr body analysis for determination of sex using light and fluorescent microscopy.	Molecular analysis	India	n.d

Table 2 - Legend: M/F - Male/Female; n.d - not determined; pm - Postmortem; BP- Before Present

There are 13 articles out of 23 that study the various methods for sex evaluation based on morphologic traits whether it is the evaluation of teeth, the mandible (hard tissue) or lip print and rugae patterns (soft tissue), corresponding to 56,5% the articles analyzed of this study (table 3).

Table 3 - Detailed information on selected studies applying morphologic methods for sex evaluation, regarding the number of Individuals, age, population origin, sample type, methods used and Sex estimation.

Study	Included subjects Total, m/f*	Age range (years)	Elements Examined	Methodology	Type of study	Population Origin	Sex estimation accuracy Mean (%)
(20) Alias AI et al., 2018	48 M48/F31	18-74	Mandibules	CT scan.	Orthometric analysis	Kuala Lumpur	78.5%
(23) Saraf A, et al., 2011	120 M60/F60	22-26	Rugae patterns	Palatal rugae classifiecation according to Thomas & Kotze and Kapali. Statistics: Chi-Square test Logistic regression analysis (LRA)	Soft tissue analysis	India	99.2%
(29) Acharya AB et al., 2008	123 M65/F58	19-28	Permanent dentitions	MD and BL measurements of all teeth using a digital calliper. dental indexes were calculated. Univariate and multivariate statistics	Odontometric analysis	Nepal	69.8–81.1%
(17) Macaluso, P. J.	235 M130/F105	12-78	First and second maxillary molar	Measurements of cusp diameters Univariate and multivariate discriminant function equations	Odontometric analysis	South Africa	58.3%- 73.6%

2010							
(16) Singh. et al., 2015	100 M45/F55	20-30	Mandibular canines	Measurement of mandibular canine teeth and calculation of the MCI Independent sample t-test	Odontometric analysis	India	85.5%
(30) Maji A. et al., 2018	60 M30/F30	20-40	Mandibular canines	Intercanine distance measurement Kruskal–Wallis test computer-assisted method	Odontometric analysis	India	n.d
(32) Carvalho SP et al.,2013	66 M34/F32	>20	Mandibles	Two mandibular measurements for sex identification, namely the bigonial distance and mandibular ramus height"	Orthometric analysis	Brazil	76.47% (Male) 78.13% (Female)
(22) Randhawa, K. et al., 2011	600 M289/F311	3 groups: 1: 1-20 2: 21-40 3: 40>	Lip prints	Cheiloscopy - Tsuchihashi classification Chi square test	Soft tissue analysis : Cheiloscopy	India	Group 1: 58.67% 2: 76% 3: 61.33%
(36) Samatha K. et al., 2016	120 M60/F60	18-45	Mandibular ramus	Mandibular ramus measurements Discriminant function analysis. Digital Panoramic and Cephalometric System. Master View 3.0 software	Orthometric analysis	India	56.5%
(15) Kiran CS, et al., 2015	120 M60/F60	15-40	Canine	The maximum mesiodistal widths of left mandibular canines. Discriminant function analysis. A cross sectional study	Odontometric analysis	India	72.5%
(21) Singh J, et al., 2012	100 M50/F50	18-30	Lip print/ Mandibular canine index	Cheiloscopy_ classification by Tsuchihashi. Determination of the MCI index. The discriminant functional analysis was used to arrive at the percentage accuracy of sex determination based on cheiloscopy (lip prints) and MCI.	Odontometric analysis/ Soft tissue analysis : Cheiloscopy	India	MCI: 85% Cheiloscopy: 55%

(9) García-Campos C. et al., 2018	69 M36/F33	20-55	Mandibular canines	The teeth were scanned, and three-dimensional (3D) measurements (volumes and surfaces areas) were obtained. Finally, a discriminant function analysis was applied.	Odontometric analysis	Spain, South Africa and Sudan	92.30%
(37) García-Campos C. et al., 2018	56 M29/F27	>6	Maxillary canines	The teeth were scanned and three-dimensional (3D) measurements (volumes and surface areas) were obtained. In addition, a discriminant function analysis was applied.	Odontometric analysis	Different geographic origins.	92.3%

Table 3 - Legend: M/F - Male/Female; n.d - not determined; CT - Computerized Tomography; MCI - Mandibular Canine Index

5) DISCUSSION

Several factors can change the appearance of bones and tissues; therefore, the identification of skeletal remains is not always simple, particularly when dealing with fragmented, decomposed, mixed, or incinerated mortal remains (1)(4). In these circumstances, teeth may be the only materials available for determining human identification as they are relatively resistant to decomposition (3)(4). In the absence of antemortem information that allows for primary methods, such as DNA profiles and dental records to be used, the reconstruction of the biologic profile is key to allow for a positive identification of individuals and sexual estimation becomes a parameter of great importance (3). A careful analysis of the different dental methods for sex evaluation, that can be broadly divided into molecular and morphologic, is therefore especially important to guide the choice of methodology in a forensic context.

1. Molecular analysis

The molecular methods for sex evaluation include DNA analysis and the detection of cellular Barr bodies. DNA plays a crucial role in Human identification and provides reliable and definitive results. For sex evaluation two genes are used: the AMEL gene, which is present in both X and Y chromosomes and SRY gene, specific of the Y chromosome. Barr bodies, which are present in female cells, constitutes an interesting alternative analysis for sex determination, that nevertheless needs to be further validated (14).

1.1 AMEL gene

Teeth are made up of enamel, the toughest tissue in the body, which acts as a shield for the DNA present in pulp tissue, that is particularly when the tooth is exposed to harmful conditions.(8)

The key protein part of enamel's organic matrix is amelogenin, which has genes on both the X and Y chromosomes : AMEL X and AMEL Y genes (9). On the X chromosome, the

amelogenin gene has a 6 bp deletion in the third intron of AMEL X that is not present in AMELY. Therefore, PCR products of AMELX and AMELY can be discriminated from one another using primers flanking a 6 bp deletion. This PCR test frequently amplifies a sequence that Is 106/112 base pairs long In the X and Y chromosomes, respectively. Females (X,X) have two similar AMEL genes (with the same size- 106 pb) while males (X,Y) have AMEL genes with different sizes (one 106pb and one 112bp) and this difference can be used to determine sex.(2,8,10). However, based solely on analysis of the amelogenin gene, an AMELY-deleted male sample, would be erroneously considered female (11).

1.2 Sex determining region "Y" gene (SRY)

The Y chromosome's Sex-determining Region (SRY) is a gene that controls the production of male traits. It is therefore responsible for the gonadal sex of the individual. The SRY gene is located on the short arm of the Y chromosome. (10)

The Y-PLEX 12 system allows for the simultaneous replication of eleven polymorphic short tandem repeat (STR) loci on the Y chromosome as well as amelogenin, which can be used in forensic and male lineage recognition situations. Amelogenin offers sex identity results and acts as a PCR internal regulator (11)

In some syndromes, maternal-fetal micro-chimerism, and dissimilar sex between donor and recipient during transplantation, false positive findings may occur (chimerism). (10)

In transplacental chimerism, (where the mother is pregnant with a male fetus, and cells exchanges happened), SRY genes can be found and migrate into the mothers organs and persist for decades which can implicate a wrong estimation.(10)

There are multiple methods to profile the Y chromosome using DNA (pulp or dentin are the best sources of DNA); Y-Chromosome analysis, Chromosome Y specific STR, Commercial kits of Y STR analysis.(11)

1.3 Barr bodies

During early embryonic growth, the chromatin materials reflect inactivation of one of the X chromosomes in each somatic cell in females forming Barr bodies. These structures, (also known as sex chromatin or X-chromatin) are tiny intranuclear bodies that stain heavily with nuclear dyes. (13) Barr bodies can be visualized in the cell nucleus using multiple special staining techniques, such as Papanicolaou dye or immunofluorescence. In this cytologic method, 40% female cells with positive Barr bodies are referred to as chromatin positive, while male cells are referred to as chromatin negative (<3%). Negative findings may occur under certain pathological circumstances and are linked to differences in the size and form of Barr bodies (14) (6).

Although an interesting method, in forensic contexts several aspects should be further addressed, namely the conditions of the tissue analyzed and the timeframe postmortem where it can be used. Further methods for Barr bodies- specific staining, should also be tested.

2. Morphological analysis

Morphological analysis includes intra and extra oral observations; hard tissue studies (odontometric and orthometric methods) and soft tissue studies (cheiloscopy, palatoscopy)

2.1 Hard tissue analysis

2.1.1 Odontometric methods

Odontometrics is referred as the measurement and analysis of tooth size which are essential in anthropology because they are known to demonstrate substantial sex dimorphism.(15) The different methods involve the use of dental indexes, usually the

mandibular canine index (MCI) (16), and also mesiodistal (MD) and buccolingual (BL) measurements. Distinct tooth morphology Is also analyzed as part of this process.

Both mandibular canine index and permanent maxillary molar exhibited significant sexual dimorphism In Independent studies (17), (18). When measuring inter-canine distance and width there was a major difference between males and females. Interestingly, in a Indian population, the left permanent mandibular canine teeth seemed to have greater sexual dimorphism than the right permanent mandibular canine teeth. (16)

A study performed by García-Campos and coworkers revealed that sexual dimorphism in canine size may be caused by males possessing more dentine, while enamel volume does not play a major role in total tooth size dimorphism.(9) However, it is not possible to establish a single value applicable for all populations and it applies only to adult individuals.(18)

2.1.2 Orthometric methods

Orthometric is referred as the dimensions and morphology of skull and mandible with different traits and parameters; angle, linear distance gonion right- menton- gonion left or the difference between the mandibular ramus's most anterior point and a line linking the condyle's most posterior point, the most accurate being the discriminant analysis. (19) (20)

The form and scale of the mandible have been used to determine sex, and male bones are normally larger and more resilient than female bones. Female mandibles have less prominent muscle marks, an inverted gonial angle, and a pointed chin, while male mandibles have prominent muscle markings, an everted gonial angle, and a squared chin. (20). Interestingly, this study used CT scans, which provides accuracy to the parameters evaluated and is also an effective method to store the Information collected, which can be valuable in a forensic context.

2.2 Soft tissue analysis

2.2.1 Cheiloscopy

Cheiloscopy is the analysis of lip prints which are, as fingers prints, unique for each individual. Several classifications of lip patterns have been proposed the most use is the one done by Tsuchihashi as follows;

"Type I: complete straight grooves; Type I ': partial straight grooves; Type II: branched grooves; Type III: intersected grooves; Type IV: reticular grooves; Type V: undifferentiated grooves"(21). (see Figure 2 in the Annex)
Vahanwala has proposed a classification of the dimorphism lips patterns, that was applied In an Indian population, as follows: *"Type I, I ': Pattern dominant – female Type II: Pattern is dominant – female Type III: Pattern present – male Type IV: Male Type V: Varied pattern – male Same patterns in all quadrants–female*". (6).
The lip print analysis Is more reliable for determining sex in middle aged individuals (20-40 y.o), sex evaluation is unreliable in the younger and older age groups because the lip maturity in the young age is reach between 14 to 18 years old. For the older, this is due to the development of wrinkles on the neighboring skin and the thinning of the lips as people become older, all of which influences the lip patterns. When people become older, their lips and perioral region lose volume, and their lip anatomy becomes less defined. With age, the inter-commissural gap grows longer, while lip height declines.(22) The age differences in the scale, shape, and skin covering the lips can affect lip print patterns.(22)

2.2.2 Rugoscopy

Rugoscopy is the study of the different patterns on the palate that can be used in human identification. The shape of the palatal rugae has been shown to be extremely unique and stable throughout life. The length, form, direction, and unification of rugae patterns are used to classify them.(6) The rugae's anatomical location inside the oral cavity (surrounded by the cheek, mouth, tongue, and buccal pad of fat) provides protection in the event of damage or incineration, therefore it's an interesting method for necro identification. (23)

Calcorrugoscopy is the overlay print of palatal rugae in a maxillary cast. The rugae pattern in males and females has been related in many trials. According to a survey of the Japanese population, females have less rugae than males.(6) In terms of rugae formation, females had a statistically higher number of converging rugae, whereas males had a statistically higher number of circular rugae.(23)

3. Comparison of the available methods for sexual estimation

In this study, amelogenin is the most utilized gene for sex determination analysis (7/23) articles). An amelogenin sequence Is usually amplified by PCR (Polymerase Chain Reaction) which shows exceptionally reliable results (75%-100% correct identification of the samples analyzed in these studies). Amelogenin peptides can also be studied with nanoflow liquid chromatography mass spectrometry as shown (8) with approximately 80–95% accuracy. SRY is also used in the genetic studies, and when using several markers, the process of sex determination seems to be more accurate (11).

In the odontometric analysis of our study, mandibular canine is the tooth mostly used (6/13 articles) and that shows the most sexual dimorphism (72,5-92.3% accuracy). For this reason, the mandibular canine index (85% accuracy in an Indian population) is a method frequently used, however, it is population dependent.

The use of mandibular ramus could be an Interesting for sexual diagnostic mainly because of the numerous parameters that may be analyzed (3 morphologic and 9 morphometric parameters observed), 56,5% to 78,5% accuracy depending on the study. Mandibular molars seem to have a more modest accuracy in sex evaluation studies (58.3%- 73.6%).

In a study performed in India, rugae patterns (classification given by Kapali et al.) showed a very high accuracy In what concerns sex determination (99,2%) (23). However, there are contradicting results particularly since it is not possible to establish a single value applicable for all populations. Therefore, these results are particularly good but can only be reliable in a certain population, using a specific protocol.

Lip print patterns categorized according to Tsuchihashi's classification (developed using natural dye (vermilion) and lysochrome (Sudan Black II) dyes) showed an accuracy of 55%-76% depending on the study (21) (22) (35). In "*Sex determination using cheiloscopy and mandibular canine index as a tool in forensic dentistry"* (21); the findings of this investigation indicated that MCI is more accurate than cheiloscopy in determining sex.

The effective identification of the individual requires a comprehensive understanding and use of the applicable evidence from the forensic scene. In mass disasters for example, some tissues may not be available for analysis, such as soft tissue, and only hard tissue remains. In this case, soft tissues analysis is of no use and hard tissues analysis is appropriate. For the same reason, orthometric analysis is used when bones are relatively analyzable, if the bones are not in good enough condition, odontometric methods (and molecular analysis) is pertinent even though, as explained above, it is not possible to establish a single value applicable for all populations and should be used only in adults. (18)

DNA-based sex determination approaches are a safer way for sex determination, and real time quantitative PCR techniques, much more efficient and accurate that the traditional PCR, as they allow for the amplification from minimal amounts of DNA (6). However, the analysis can go wrong in situations such as chimerism where a thorough examination may be required to identify a sample from a chimera. (10) Furthermore, DNA techniques may not always be used due to DNA degradation or technical difficulties, depending on the forensic circumstances. Microscopic techniques, such as using Barr bodies to determine sex, could be interesting and technically simpler but display decreasing accuracy as time passes after death.(14). Also, further studies are needed to validate a protocol that can be applicable to a forensic setting. Interestingly, in archeological subjects, using peptides retrieved from tooth enamel may be an efficient alternative method to DNA sequencing, since it is expensive, time-consuming, and often difficult to obtain, due to the poor quality of the DNA obtained in samples from hundreds to thousands of years old (8).

When soft tissues and hard tissues are both available for analysis, the mandibular canine index is more relevant than the analysis of other teeth such as molars (6) and much more efficient that techniques such as cheiloscopy (21) or palatoscopy (23). As seen before,

sex evaluation using odontometry is difficult in the subadult population and when using soft tissue analysis, one of the main drawbacks is that it cannot be used at any point of decomposition of corpses, restricting its use to an early post-mortem analysis.(5) Furthermore, sex differences associated with lip prints and palatal rugae, seem to be affected by the population of study and there are several different methods of classification. As a result, lip prints and palatal rugae, have a limited use for sexual estimation, but in addition to the dentition, may be useful as complementary methods, to help determine an individual's identity in specific populations.(6)

6) CONCLUSION

In forensic odontology, sex determination may be performed using either morphological (hard and soft tissue) or molecular analysis. The examination of DNA from harvested pulp or the tooth itself through the AMEL gene and SRY gene should be used whenever possible. Other molecular methods such as Barr body analysis could be useful, although for a limited time following death, and further studies are needed to confirm its practical usefulness in forensics. Odontometry and the analysis of teeth and morphological structures associated, can provide the useful information regarding the individual's sex and the reconstruction of a biological profile. Odontometric methods can be used in adults, namely when applied to dimorphic teeth such as canines. This analysis can be valuable, especially in circumstances where DNA techniques are not possible, due to DNA degradation of other technical issues. Other teeth, such as molars could be interesting alternatives when the canines are absent, although further studies in different populations are needed to validate these measurements. The use of cheiloscopy and palatoscopy for sexual estimation, although interesting is quite limited: it depends on the population, there are no universal standards for the classifications and furthermore, they can only be used for a limited time, following death. The use of CT scans to evaluate damaged and/or unknown mandibles, can be quite an interesting

method for sexual evaluation and further studies using this technique are expected to be further developed and used in the future.

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7. APPENDIX

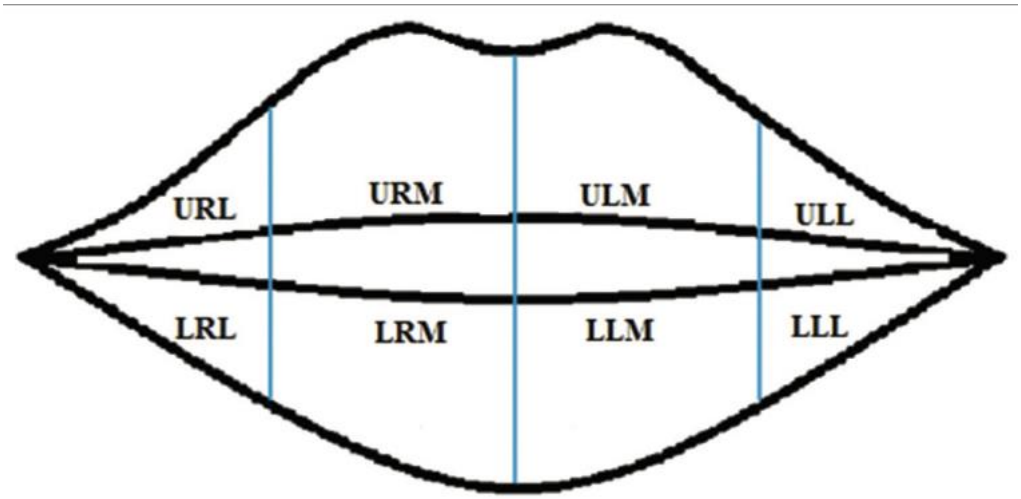

Figure 1 - Diagram showing the quadrants and segments of the lip. URL: Upper right lateral, URM: Upper right medial, ULM: Upper left medial, ULL: Upper left lateral, LRL: Lower right lateral, LRM: Lower right medial, LLM: Lower left medial and LLL: Lower left lateral (38)

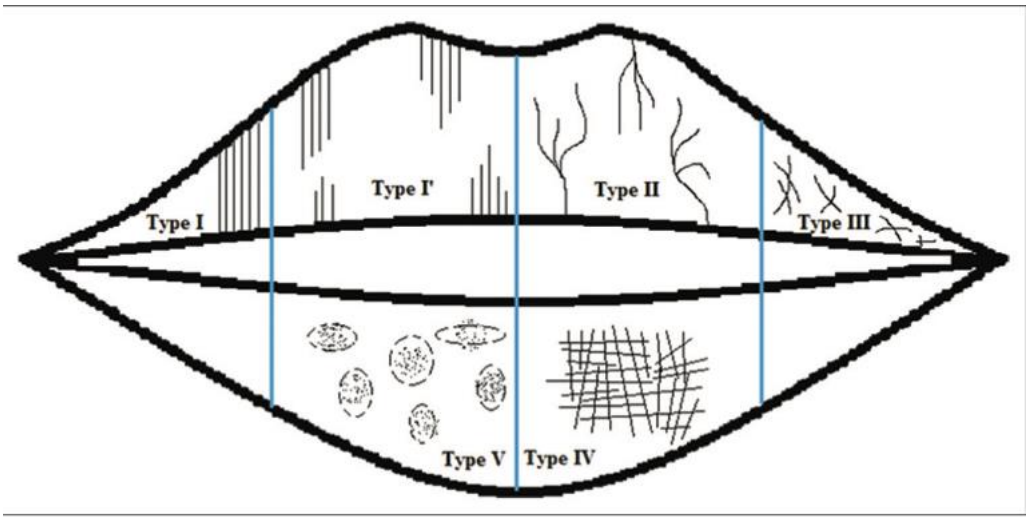

Figure 2 - Diagram showing the Tsuchihashi classification of lip prints. Type I vertical grooves, Type I' partial length groove of Type I, Type II branched groove, Type III intersected groove, Type IV reticular groove and Type V other patterns (38)

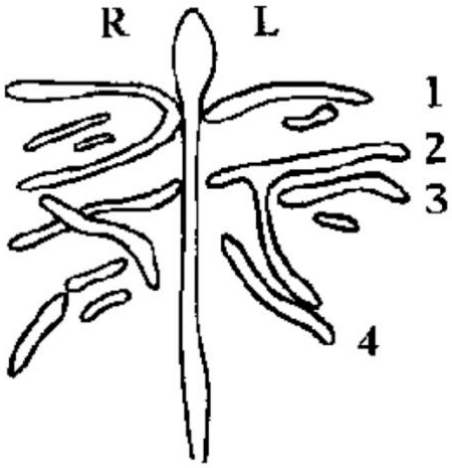

Figure 3 – Numbering of palatal rugae; four main types: punctuate, straight, curved and composite. classification given by Kapali et al.
These classifications include number, length, shape and unification of rugae (divergent/convergent). Three length categories were formed: “Primary rugae: (A-5 to 10 mm; B-10 mm or more) - Secondary rugae: 3-5 mm - Fragmentary rugae between 2-3 mm. (All rugae more than 1 mm long were recorded under the fragmentary rugae category).” (23)

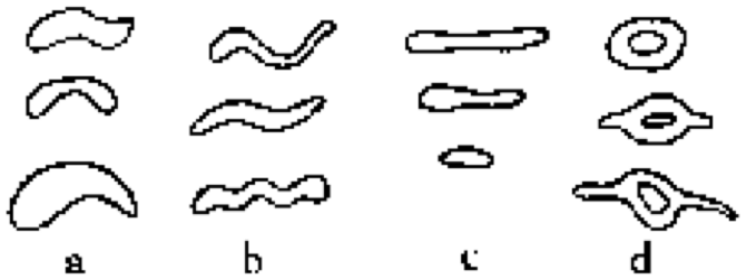

Figure 4 – Various shape of rugae; a, curved. B, wavy. C, straight. D, circular. Classification given by Kapali et al. “The curved type had a simple crescent shape which curved gently. Evidence of even the slightest bend at the termination or origin of rugae led to a classification as curved. The basic shape of the wavy rugae was serpentine. To be classified as circular, rugae needed to display a definite continuous ring formation.” (23)