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A forensic review on sex determination methods for teeth

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ABSTRACT

Teeth are widely used, durable and best-preserved parts of the human body used in forensic and anthropological investigations. To establish the biological profile from unidentified remains, tooth is said to be an excellent piece of evidence in the incidents such as bomb blast, terrorist attacks, air plane crash and mass disasters etc. where dead bodies recovered are dismembered and mutilated beyond recognition. Dental evidence considered to be valuable for sex determination in case of critical body conditions, insufficient or unavailable body parts. The various methods used for sex determination from teeth mainly includes visual/ clinical, microscopic and advanced methods. This review paper discussed the challenges and various methods used for the sex determination from teeth since 1973 to 2020.

Keywords: Sex determination, Teeth, Forensic odontology, Biological profile, Forensic investigation

INTRODUCTION

"Forensic odontology is a branch of dentistry which deals with the proper handling and examination of dental evidence and the proper evaluation and presentation of dental findings in the interest of justice" (Goldman, 1982). The identification of human remains that rely on the individual characteristics present in the teeth of different individuals, is the primary goal of forensic odontology. In incidents such as tsunamis, landslides, earthquakes, air plane crashes and terrorist attacks etc. where highly mutilated and dismembered dead bodies are recovered and which are beyond recognition; this discipline plays a vital role in personal identification (Hinchliffe, 2011; Krogman and Iscan, 1986).

Personal identification is carried out with the help of the gender, age, stature and race of an individual. Gender estimation being a crucial part of personal identification must be determined first. This is often an imperative step in establishing the personal identity of an individual, as the age and stature estimation depends on the gender of an individual (Zoya and Renu,

2018). In addition, sex determination may cut the number of possible matches in half (Macaluso, 2010).

Teeth are extensively utilized in forensic and anthropological investigations to determine the sex of human remains, as it is the best-preserved part of the human body (Jeevan *et al*. 2011, Tarvido *et al*. 2011). Teeth enamel may be a unique entity among all mineralized tissues due to the presence of high mineral content in it (Higgins and Austin, 2013). Therefore sex can be determined by dental feature comparison and tooth dimensions (Chinagorom *et al*. 2018).

For sex determination, visual, microscopic and advanced methods are mainly used. In visual methods, morphological dimensions such as tooth size, root length and crown diameter etc. are considered for male and female sexes. In the microscopic method, sex chromatin while in advanced method PCR and enamel protein (amelogenin) have been used for the sex determination (Monali *et al*. 2011)

The present review is based on the various methods used for sex determination from teeth during 1973 to 2020, for which the Pubmed, Google scholar databases, research articles and reviews were screened as per the title, abstract and full text.

Methods for sex determination from teeth:

Various methods are used by researchers for the determination of sex from teeth, which includes a visual method, microscopic and advanced methods shown in the table. 1.

Visual method:

Tooth size:

Sex determination utilised in this method depends on the sexual dimorphism of the teeth size (Krishan *et al*. 2015). The tooth size or odontometrics are always population-specific and cannot be used the same measurement across the world due to the extensive influence of the environment on teeth (Dinakaran *et al*. 2015). A study conducted on American Negroes evaluated the mesiodistal crown dimension of permanent dentitions and reported that male teeth were larger as compared to female teeth for each type of tooth in both arches (Richardson and Malhotra, 1975).

As per the study carried out on the Nepalese subject, in which sex differentiation was based on buccolingual (BL) and Mesiodistal (MD) dimensions. The higher accuracy was obtained when both the dimensions used concurrently in dental sex assessment (Acharya and Mainali, 2008).

Root length and crown diameter:

Root length and crown diameter used for sex differentiation are neither permanent nor always reliable but can be population specific. This method relied upon the morphological features of tooth crown and root because of the dissimilarity in the thickness of enamel (Vodanović and Brki, 2012).

A study on mandibular permanent teeth using an optical scanner and radiogrammetric measurements yields 80% accuracy in sexing when root length and crown dimensions used in combination and discriminatory Effectiveness of 87% with mandibular teeth alone (Garn *et al*. 1978).

Canine dimorphism:

Among all teeth, a canine is one of the toughest and stable teeth because of its shape, structure and root length. In addition, canines are situated in the mandible and maxilla and have a single cusp which makes them less exposed to devastation or cavity formation. Therefore, canines have more forensic significance (Zoya and Renu, 2018).

Several methods were studied for canine teeth dimensions, such as Fourier analysis, Moire topography and measuring linear dimensions of teeth like mesiodistal width, bucco-lingual width and inciso-cervical height. (Anderson and Thompson, 1973, Garn *et al*. 1967, Monali et al. 2011, Rao *et al*. 1988).

The mandibular canine width and intercanine distance was measured found to be more for males as compared to females in white children from Burlington growth centre and found 74% of cases showed admissible accurate sex differentiation (Anderson and Thompson, 1973).

For sexual dimorphism, a study conducted in different ethnic groups of Ohio measured the mesiodistal width of

canine teeth and found that the mandibular teeth showed a higher degree of sexual dimorphism as compared to maxillary canine. (Garn *et al*. 1967)
Sex determination using mandibular canine index showed 84.3% accuracy in males while 87.5 % in females in south Indian population (Rao *et al*, 1988) whereas contrary results were obtained in Devangere (India) population which showed 83.3% accuracy in males and 81% in females (Yadav *et al*. 2002).

Using maxillary and mandibular canines and mandibular second molar, sex determination was accurately established in 77% of cases (Iscan and Kedici, 2003). Sex differentiation using canine dimorphism reported that male having greater canine dimensions than female. (Zoya and Renu, 2018).

Tooth morphology and sexing :
The most widely recognized morphological feature of the tooth which separates men from women is the deflecting wrinkle which epitomizes a variety of the medial ridge on the mesio-lingual cusp of the primary lower molar, wherein the edge deviates towards the disto-lingual cusp. The presence of this wrinkle is a trait that is only seen in men. Among the obtained morphological features, which separate men from females, tooth abrasion is the most significant. Because of more potent masticatory muscles and greater masticatory forces, men suffer more tooth wear as compared to females, which can also additionally cause a reduced tooth crown height. (Vodanović and Brki, 2012).

In human dentition, distal accessory ridge (a non-metric feature on canine) is the extreme sexually dimorphic crown trait, which showed considerably higher frequencies in males and greater pronounced expression in females (Scott and Turner, 1997).

Dental index:
For sex determination, tooth proportions have been suggested in addition to the tooth size. Aitchison gave the formula for calculating 'Incisor index' (Ii) as,

Ii= (MDI2/MDI1) × 100
Where,
MDI2 = Maximum mesiodistal diameter of the maxillary lateral incisor
MDI1= Maximum mesiodistal diameter of the central incisor

Incisor index is higher in males, verifying the proposal of Schrantz and Bartha that the lateral incisor is less than the central incisor in females (Dinakaran *et al*. 2015).

Rao *et al* proposed the "Mandibular canine index" and have given a precise sign of sex in the Indian population. Utilizing the mesiodistal (m-d) measurement of the mandibular canines, these researchers derived the formula as,

Mandibular canine index = [(Mean m-d canine dimension + (Mean m-d canine dimension in female + standard deviation [SD]) in males – SD)]/2.

The greatest probable mesiodistal dimension of mandibular canines was 7.1mm in females, obtained by utilizing the 'Mandibular canine index" formula. A similar dimension is more in males. The success rate of sex determination using this formula was nearly 89%. However, it is close to 100% when utilizing the pelvis and skull. Therefore, sex determination through odontometrics is relatively poor (Dinakaran *et al*. 2015, Rao *et al*. 1988).

Odontometric difference:
The odontometric difference between male and female is usually explained because of more genetic expression in males. There may be a coincidence between male and female tooth dimensions, and this amount to precise diagnosis of sex challenging, in any event, for experienced dental specialists. They signify that when the whole available teeth are utilised then success is greater (Iscan and Kedici, 2003).

Male have bigger teeth as compared to females and this dimension is a good gender indicator compared to the buccolingual dimension. Because of the complexity in estimating the mesiodistal measurements as a result of close proximal contacts, there might be an inconsistency in its estimation. So encompassing both the dimensions for the determination of sex would be more effective and authentic (Lakhanpal *et al*. 2013).

In gender determination, between maxillary and mandibular teeth, the mesiodistal dimension of the

mandibular canine exhibits more significance, when tooth dimensions are taken into account for gender prediction (Hemani *et al*. 2008).

Microscopic method:

Using Barr bodies:

Sex can likewise be determined by the study of X & Y chromosomes in the cells which are not going through active division. The presence or absence of X chromosome can be examined from buccal spreads, skin biopsy, blood, ligament, hair root sheath, and tooth pulp. After death, it continues for variable periods relying on the humidity and temperature of the surrounding climate. X chromatin and intra-nuclear structure referred to as Barr body as it was first found by Barr and Bertem (1950). It is available as a mass ordinarily lying against the nuclear membrane in the females (Barr *et al*. 1950).

A study showed that sex determination from pulpal tissue in cadaver was valid up to 4 weeks from the study of X and Y chromosome keeping in view of a change in temperature and humidity. In addition, the mean percentage of Barr bodies was found to be 2.12 % +/- 1.41 % and 24. 92% +/- 3.74% for male and female respectively. Whereas the mean percentage of F bodies was found to be 35.64% +/- 6.49 % and 2.27 % +/- 1.30 % for male and female respectively (Das *et al*. 2004).

Sex from necrotic pulp tissue stained by quinacrine mustard utilizing fluorescent Y chromosome test for maleness and guaranteed that up to 5 weeks after a death, sex determination can be done with high accuracy (Whittaker *et al*. 1975).

In one of the study, Barr bodies and F bodies Y chromosome are kept in dehydrated pulp tissues till one year and pulp tissues preserved sex diagnostic characters when heated until 100˚c for 1 hour (Duffy *et al*. 1991).

Advanced methods:

PCR:

The arrival of the polymerase chain reaction (PCR) drasticcally changed biological science from the time it was first found (Mullis, 1990). For amplifying small quantities of adequate short target sequences of DNA utilizing sequence-specific oligonucleotide primers and thermostable Taq DNA polymerase, PCR is used (Tsuchimochi *et al*. 2002).

For personal identification teeth are utilized as they can withstand high temperatures. On account of few teeth or missing dental records, there isn't sufficient data to identify the person. The dental pulp surrounded by hard tissue is not affected by temperature, in contrast to the buccal mucous layer, salivation, and analytics (Hemanth *et al*. 2008).

As per the study conducted among Egyptian, sex was determined from dental pulp DNA. In which DYS14 and SRY genes were PCR amplified and found to be reliable for dental sex determination, no matter the condition of the teeth is sound or carious (Kholief *et al*. 2017).

A study utilised epithelial cells clinging to the acrylic removable partial denture for sex determination. A sex-determining region of the Y chromosome (SRY) gene used amplified using RT-PCR and found that sex determination was possible from the whole samples with 100% accuracy (Bharath *et al*. 2019).

Enamel protein (Amelogenin):

In human enamel, amelogenin is a major matrix protein and has different signatures i. e. having different size and pattern of nucleotide sequence in males and females. The AMEL gene that encodes for female amelogenin is situated on the X chromosome and male amelogenin is situated on the Y chromosome. This can be utilised for sex determination of remains with a very small DNA sample as the female has two similar AMEL genes whereas the male has two different AMEL genes. (Dayal, 1998).

Few studies used the amelogenin gene as a method for analysis for sex determination found that all the presented amplicons have different size as, 330 bp (X) and 236 (Y) (Chowdhury *et al*. 2018), ~1.5 kb (X) and ~1.3 kb (Y) (Dutta *et al*. 2017), 281 bp (X) and 287 (Y) (Lim *et al*, 2019), 106 bp (X) and 112 bp (Y) (Zapico and Ubelaker, 2013) in PCR amplification. In addition, two amplicons (X-Y) were shown by males whereas a single amplicon in X form was shown by females (Maulani and Auerkari, 2020). As per the study conducted on Indian children, sex determined from mesiodens by amelogenin gene found that mesiodens could function as a reliable source of DNA for amplification-based forensic methods in sex determination (Srivastava *et al*. 2017).

Sex determination by analysis of amelogenin gene from dental pulp tissue by PCR method showed that even in extreme conditions except for high temperature, teeth can be a great source of DNA and sex determination by PCR amplification of AMEL markers can be quite reliable (Chowdhury *et al*. 2018).

The identification of the amelogenin gene on burnt teeth samples analysed with Nested PCR for sex identification, found that Nested PCR of AMEL gene established to be an appropriate method for unequivocal sex determination from degraded DNA samples (Lim *et al*. 2019).

Nanoflow LCMS (Liquid chromatography-mass spectroscopy):
From peptide in human enamel which is a minimally destructive surface, sex was determined. Firstly, the tooth enamel etched by acid and later identification of sex chromosome-linked isoform of amelogenin by NanoLCMS i.e. Nanoflow Liquid Chromatography-Mass Spectroscopy (Stewart *et al*, 2017).

CBCT (Cone beam computed tomography):
Based on pulp cavity volume of upper central incisor and canine, sex estimated in a Brazilian population using cone-beam computed tomography. High accuracy obtained when a person's age was known (Andrade *et al*, 2019).

Table 1: A overview of various methods used by researchers for the determination of sex from teeth

Sr. No.	Methods	Factors/Technique used	Analysis method	References
1	**Visual method**	Tooth size	Using boley gauges, teeth were measured from the plaster cast mesiodistal crown dimension of permanent dentitions was studied.	(Richardson and Malhotra, 1975)
			Using a digital calliper, the mesiodistal and buccolingual dimensions were measured for all teeth except the third molar.	(Acharya AB and Mainali, 2008)
		Root length and crown diameter	Optical scanner and radiogrammetric measurements were used on mandibular permanent teeth	(Garn *et al*. 1978)
		Using canine dimorphism	Fourier analysis	(Minzuno, 1990)
			Moire topography	(Suzuki *et al*. 1984)
			The mandibular canine, lateral incisor, and first molar teeth were studied and was measured on a dental cast	(Anderson and Thompson, 1973)
			Mesiodistal width of canine teeth	(Garn *et al*. 1967)
			Mandibular canine index	(Rao *et al*. 1989)
			Bucco-lingual dimensions	(Iscan and Kedici,2003)
			Using mandibular canine index	(Latif *et al.* 2016*)*
			Mesiodistal width, lingual width and intercanine distance of mandibular and maxillary canine	(Zoya and Renu, 2018)
			Measured the mesiodistal and buccolingual diameters of permanent canine from an alginate impression	(Yepes *et al.* 2019*)*
		Tooth morphology and sexing	A non-metric parameter on canine called distal accessory ridge utilised to differentiates sex	(Scott and Turner, 1997)
		Dental index	Mesiodistal dimension of the mandibular canines	(Rao *et al*. 1988)
		Odontometric	Mesio-distal dimensions of permanent maxillary	(Rahul *et al.* 2014*)*

		parameter	incisors and canines	

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Sr. No.	Methods	Factors/Technique used	Analysis method	References
			Utilising mesiobuccal to distolingual (MB-DL) and distobuccal to mesio-lingual (DB-ML) widths of right maxillary canines, premolars and first molars	(Dumpala *et al.* 2014*)*
			Using six parameters of maxillary teeth inter canine width (ICW), Inter-premolar width (IPMW), Intermolar width (IMW), Left and right maxillary width (LCCW, RCCW) and Maxillary depth (MD)	(Chinagorom *et al.* 2018*)*
			Impression of the maxillary arch was taken using alginate and measurements such as interpremolar and intermolar arch width were taken using a vernier calliper	(Anjum AS and Don KR, 2019)
			Seven measurements such as canine width, intercanine width, intermolar width, buccolingual diameter, mesiodistal width, mesiobuccal distolingual diameter and Distobuccal mesio-lingual diameter were taken using alginate dental teeth using a vernier calliper	(Sharma *et al.* 2019*)*
			Mesiobuccal-distolingual (MBDL) and distobuccal - mesiolingual (DBML) measurement of the right permanent maxillary and mandibular teeth except the third molar were taken and analysed by discriminant function analysis	(Sathawane *et al.* 2020*)*
			Mesiodistal dimension of permanent teeth from right first molar to left first molar in each jaw was taken from cast	(Daniele *et al.* 2020*)*
			Mesiodistal width (distance between mesial and distal contact point) was measured for each tooth utilising dental cast	(Neves *et al.* 2020*)*
2	**Microscopic methods**	Using Barr bodies	X & Y chromosomes keeping in view the variation of temperature and humidity.	(Das *et al*. 2004)
			Necrotic pulp tissue stained by quinacrine mustard using fluorescent Y chromosome test for maleness	(Whittaker *et al*. 1975)
		Sex chromatin (Barr bodies and F bodies)	Sex chromatin (both Barr bodies and F bodies) assessed in artificially mummified and heated pulp tissue	(Duffy *et al*. 1991)
		Tooth pulp tissue	Sex determined by using fluorescence microscope by identifying Y chromosome fluorescence in dental pulp	(Veeraraghavan *et al.* 2010)
			Necrotic pulp tissue stained by quinacrine mustard using fluorescent Y chromosome test for maleness	(Whittaker *et al*. 1975)
		Sex chromatin (Barr bodies and F bodies)	Sex chromatin (both Barr bodies and F bodies) assessed in artificially mummified and heated pulp tissue	(Duffy *et al*. 1991)

Table 1: A overview of various methods used by researchers for the determination of sex from teeth

Sr. No.	Methods	Factors/Technique used	Analysis method	References
		Tooth pulp tissue	Sex determined by using fluorescence microscope by identifying Y chromosome fluorescence in dental pulp	(Veeraraghavan *et al.* 2010)
3	**Advanced methods**	PCR (Polymerase chain reaction)	Extracted DNA using Chelex method from dental pulp and sex determined from incinerated teeth with Y-chromosomal alphoid repeat and STR	(Tsuchimochi T *et al*. 2002)
			For determination of sex, PCR amplification of the alphoid satellite family utilizing amplification of X (131 bp) and Y (172 bp) specific sequences in males and Y specific sequences in females	(Hanaoka *et al.* 1996)
			DNA prepared from the hard tissue by ultrasonication and utilised PCR amplification of amelogenin gene segment for determination of sex	(Sivagami and co-workers, 2000)
			Extracted DNA from dentin and pulp utilizing a silica-based method and amelogenin gene was used for sex determination via PCR	(Zapico and Ubelaker, 2013)
			Extracted DNA from freshly extracted dental pulp using QIAamp* DNA investigator kit and DYS14 and SRY genes were PCR amplified	(Kholief *et al*. 2017)
			Isolated DNA from human extracted mesiodens and PCR carried out using pre-designed primers for amelogenin AMEL X and AMEL Y	(Srivastava *et al*. 2017)
			Extracted DNA from dental pulp and amelogenin gene was utilised for determination of sex by PCR amplification	(Dutta *et al*. 2017)
			Isolated DNA using an organic phenol-chloroform method and from the dental pulp by amplification of AMEL gene by PCR, sex was determined	(Chowdhury *et al*. 2018)
		Real-time -PCR	DNA extracted using Real genomic YGB 100 DNA extraction kit from the acrylic removable partial denture, RT-PCR used for amplification and detection of the presence of SRY gene, SRY sex-typing marker was utilised	(Bharath *et al*. 2019)
		Nested PCR	DNA extraction was done from burnt teeth and the amelogenin gene was Identified on burnt teeth through nested PCR amplification for identification of sex	(Lim *et al*. 2019)
		Nanoflow LCMS (Liquid chromatography-mass spectroscopy)	From peptide in human enamel (firstly, acid etching of tooth enamel and later identification of sex chromosome-linked isoform of amelogenin by NanoLCMS)	(Stewart *et al.* 2017)
		CBCT (Cone beam computed	Utilised CBCT (Cone beam computed tomography to estimate sex from pulp volume of upper central	(Andrade *et al.* 2019)

		tomography)	incisor and canines	

Challenges in sex determination from teeth:

1. The main challenge in sex determination from teeth is associated with obtaining and interpreting the antemortem records because occasionally due to the timeperiod there could be inconsistency while comparing these records (Ramakrishnan *et al*. 2015).
2. The identification primarily based on dental charts fails in a few instances because of the absence of ante-mortem records, so DNA typing techniques are essential for specific personal identification (Williams *et al*. 2004).
3. In real forensic cases, the sample containing degraded DNA can inhibit analysis or lessen the resolution. In this case, degraded DNA may provide little or no chance for analysis or specific conclusion (Rubio *et al*. 2009).
4. Although amelogenin is extensively used as a marker, in a few instances, deletion in the amelogenin Y region or loss of Y-DNA has been discovered despite the presence of Y chromosome. Thus, this technique in a few instances misidentified males as females, resulting in the wrong conclusions (Chaerita *et al*. 2020).

CONCLUSION

In various incidents such as bomb blast, terrorist attacks, plane crashes and mass disasters etc. When no other evidence is found at the scene of crime then teeth can be valuable evidence. Though sex determination from the teeth is uncertain it can provide a clue for individuals sex and it can be supplement with the other facts of the case and the availability of data to forensic scientist or expert. Though the sex determination through microscopic, advanced methods are expensive, time-consuming and laborious, they are found to be more useful in many incidents where only fragments of teeth were found. Whereas visual or clinical methods are simple, rapid but provide only minor details and not highly reliable as its reliability is compromised due to dental abnormalities, regressive alteration of teeth, nutritional or other external factors. Thus the significance of each method is equally important in sex determination when any of the method is impracticable due to inevitable circumstances. In the forensic cases, the expert can choose a suitable method as per the case and conditions of the dental evidences.

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