

Gender Determination by Forensic Odontologist: A Review of various methods

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Abstract: The employment of dental principles to legal issues in the interest of justice is termed Forensic Odontology. Sex determination is a branch of Forensic Odontology and it is the first step leading to human positive identification, hence very important. Sex determination can be a challenging task to forensic investigators, especially in cases of mishaps, chemical and nuclear bomb explosions and where only fragments of the body are recovered. This article aims to review the different methods available for sex determination.

Keywords: Forensic odontology, dental tissues, sex determination

I. Introduction

Forensic odontology is that subdivision of forensic medicine that deals with proper scrutiny, management and presentation of dental evidence in court of law for justice sake. One of the challenges that forensic experts might face is establishment of one's identity. The concept of identity includes a set of characteristics that define an individual. It is opined that, sex assessment being a prerequisite for identification, is the first priority in the process of identification. Skeleton remains have been used for determining the gender of an individual and has proven to give accurate results when the skeletal bones are complete. However, sex determination with the help of skeletal remains becomes a confusing puzzle for forensic experts especially when the skeletal remains are fractured or incomplete. In such cases, the use of dental remains such as teeth is an excellent piece of evidence that can be used to determine gender. Teeth being the most enduring and stable part of the body can withstand degradation from extreme conditions even after death of an individual. Hence, teeth are considered to be very crucial for identification during the investigative procedures, especially when there is lack of any other evidence. Sexual dimorphism represents a group of morphologic characteristics that discriminate gender of an individual. Among these dimorphic traits, literature indicates that tooth has been assessed in different populations for its applicability in investigations pertaining to identification. According to many authors, morphological differences of teeth can be applied to identify the gender from dental remains.¹

Sex Determination Analysis

Sex determination analysis from teeth can be done via morphological analysis or molecular analysis.¹⁻³

Morphological Analysis

Hard tissue analysis

Tooth size

Odontoscopy, from dental anthropology point of view seeks to observe records, analyze and understand the behavior of the expression of coronal and root morphology of human teeth. Studies have revealed that measurements of the mesiodistal and buccolingual dimensions of teeth are excellent indicators of sex being the most easiest and reliable method to analyze sexual dimorphism. The mesiodistal dimension is the greatest distance between the contact points on the proximal surfaces of the crown and buccolingual dimension is defined as the greatest distance between the labial or buccal surface and lingual surface of the tooth crown.⁴⁻⁶ It is generally accepted that male poses larger teeth than the opposite sex; hence, it is no surprise to many authors that the latter has lower mesiodistal and buccolingual dimensions.⁷⁻⁸ Nevertheless, there has been a debate on which dimension (mesiodistal or buccolingual) give better results. Several researchers (Garn *et al.*, 1988, Iscan and Kedici, 2003) indicated that the buccolingual dimensions are more accurate because of the great difference obtained between male and female.⁹ In contrast, earlier studies argue that mesiodistal dimensions are more accurate instead.⁷ Even though these measurements are used for determining sex, they present certain drawbacks. A major disadvantage of mesiodistal measurements is that they are susceptible to proximal wear and attrition, which may reduce the dimension and render them of little use in forensic investigations. While buccolingual

surfaces are not altered by proximal wear, they are modified by marked attrition and may also be affected by large deposits of dental calculus. It is vital to note that tooth size is under extensive influence of the environment. Therefore, Gomez (2013) and Iscan (2003) stressed that such measurements are population specific, and do not apply to the world at large.^{7,9}

Canine dimorphism

Canine teeth measurements (mesiodistal width, buccolingual width and inciso-cervical height) have been studied by several methods such as Fourier analysis (Minzuno, 1990) and Moiretopography (Suzuki et al., 1984) to mention a few. Mandibular canines considered to be the “key tool” for personal identification are well known for exhibiting the greatest sexual dimorphism.¹⁰

Several theories have been given to explain canine dimorphism.^{12,7,5} According to Acharya et al., (2007), Y chromosome produce slower male maturation. Furthermore, the long period of amelogenesis process in male contributes to this observation. As a result, male have thicker enamel than female.¹⁰⁻¹¹

Bossert and Marks (1996) in their study reported that evaluation of permanent mandibular and maxillary canine teeth for sex determination has certain advantages in that they are the least extracted teeth, less affected by periodontal disease, last teeth to be extracted and has a higher chance to survive severe trauma.¹² A study by Anderson and Thompson (1973) revealed that mandibular canine width and inter-canine distance measurements are greater in males than in females. These measurements permitted an accurate differentiation of gender up to in 74%.¹³ Garn et al., (1988) measured the mesiodistal width of canine teeth in different ethnic groups and found that the mandibular canine showed a greater degree of sexual dimorphism than the maxillary canine.¹⁴ Similarly, Rao et al., (1989) reported that the mesiodistal width of mandibular canines was significantly greater in Indian males than females.¹⁵

Dental index

Tooth proportions have been recommended for gender assessment apart from complete tooth size. Hence, Aitchison (1964) put forth the “incisor index” (Ii), calculated by the formula:

$$Ii = \left[\frac{MDI2}{MDI1} \right] \times 100,$$

where MDI2 is the maximum mesiodistal diameter of the maxillary lateral incisor and MDI1 is the maximum mesiodistal diameter of the central incisor. Aitchison found out that this index tends to be higher in males.¹⁶

Rao et al., (1989) suggested another index, the “mandibular canine index” which gave an accurate indication of sex in the Indian population with an accuracy of 89%. Using the mesiodistal (m-d) obtained the formula:

$$\left[\frac{\text{Mean m-d canine dimension} + (\text{Mean m-d canine dimension in female} + \text{standard deviation [SD] in males} - \text{SD})}{2} \right]^{17}$$

Tooth shape

Non metric features may be defined as traits that are recorded by visual observation in terms of presence, absence or degree of development. Scott and Turner¹⁸ suggested that genetic and environmental factors led to evolution of characteristic features of different population groups. Non metric features such as distal accessory ridge on canine and number of cusps in mandibular first molar may be used to determine gender. Scott and Turner emphasized that the distal accessory ridge of the canines is often present and more prominent in male than in females. Anderson and Thompson have reported greater incidence of four cusps on the mandibular first molar in male as compared to females.¹³ Similarly, Rao et al., (1988)¹⁵ carried out a study on South Indian population and came to the same conclusion as Anderson and Thompson. Moreover, Anderson and Thompson opine that evolutionary trends have led to the reduction in the number of cusps as well as towards general shrinkage of the lower face, with male seemingly resisting this drift.¹⁸

Orthometric method

This method involves the use of the skull, mandible, frontal and maxillary sinuses as a tool for sex determination. Many studies reveal that sexing can be accurately determined up to 95% using various features of skull and mandible. However the gender determination using skull alone is not reliable well until puberty, hence, the need to combine skull features, mandible features and sinuses dimensions to bring about more accurate results. The mastoid, supraorbital ridge, nasal aperture, size and architecture of skull, zygomatic extensions and mandible gonial angle play a major role in gender determination. Anthropological studies have revealed that an accuracy of 94% in gender determination could be achieved using only these traits.^{3,4}

Skull: The skull of male and female vary in shape principally due to size. Female skull is made up of much lighter bones than males, with smooth surfaces. Male has a low and sloped frontal bone, while the female's frontal lobe is higher and more rounded. The male's eye orbits are rather square shaped, lower,

somewhat smaller with rounded superior margins. On the contrary, the female eye orbits are more circular, higher and larger with very sharp superior margins. Zygomatic bones of males are greater than that of females.¹⁹ Sangvichien et al., (2008) used the cranium and mandible of Thais people to assess how accurate and reliable the bones could be regarding sexing. Using two methods (Krogman's cranioscopy and modified Krogman's cranioscopy) the author reports that using Krogman's cranioscopy has a high accuracy in sexing. Sex in males was accurately determined up to 95.5%, 82.9% for females and 91.1% overall.²⁰

Mandible: the mandible, also known as the lower jaw bone probably plays a major role in sex determination by a Forensic Odontologist. The difference in the development of the musculoskeletal system, specifically muscles of mastication attached to the jaw bone of male and female is blamed for shaping the mandible differently. In a study carried out earlier on the angle of the mandible on a mixed population revealed that the angle varied between 110° and 140° in adults of both sex. It was also concluded that people who retained their teeth are not prone to an increase in the angle with advancing age.²¹ A digital radiographic study carried out in 2012 on the mandibular ramus indicated that the ramus breadth is the best parameter for sex determination.²² Thakur et al., (2013) conducted an anthropological study on the mandibular angle and height of the ramus to test their role in sexual dimorphism, and results showed both these parameters are greater in males than in females.²³ Sharma et al., (2016) recently conducted a study on an Indian population and used parameters such as the length of body of the mandible, angle of the mandible and minimum ramus breadth as chief parameters for sex determination. The author reports an accuracy of 60% in sexing using those parameters only, however, statistical analysis indicated lack of any significant differences between the male and female gonial angle.²⁴ A recent study²⁵ reports that there is no correlation between gender and gonial angle, although the angle increases in the elderly mandibles, especially if the dentures are not worn. This finding contradicts many studies that report otherwise.

According to the Textbook of Anatomy, male mandibles are noticeable by the squared shape of the chin. Female chin structures are more often pointed or 'V' shaped; however, they can display a square form at times. The analysis of the mandible alone is less reliable in determining sex and should be used in correspondence with other indicators.²⁶

Paranasal sinuses (maxillary and frontal): Surrounding the nasal cavity is a group of four paired air-filled spaces, the paranasal sinuses. Amongst the sinuses, the maxillary sinuses positioned beneath the eyes in the maxillary bones are the biggest. The maxillary sinuses reach their mature sizes at the age of 20 years after complete development of the permanent teeth. During adulthood, their shapes and sizes are altered especially due to loss of teeth. After the maximum growth of an individual, reduction in the volume of the maxillary sinus is observed in both genders. Authors report that the loss of minerals in the bone matrix that surrounds the maxillary sinus leads to its contraction.²⁷ Jasim and Al-Taei, (2013), conducted a study on computed tomographic measurement of maxillary sinus volume and dimensions in correlation to age and gender among individuals with dentate and edentulous maxilla. Results showed that the maxillary sinuses in males are larger in volume and wider in width than that of opposite sex; also, the depth and height are higher in males compared to their counterparts. In addition, no significant difference was found between the left and right side. Furthermore, the mean right and left maxillary sinus volume and dimensions showed no significant differences between dentate and edentulous group except the measurements of height were considerably higher in edentulous group than that of dentate group.²⁸ Maxillary sinus volumes and dimensions, however, show a wide range in different studies that may reflect the influential effects like human variability. Some authors studied the volumetric measurements and anatomical variations of paranasal sinuses in dried skulls of Africans (Nigerians) and found that the maxillary sinus average volume on the right was (11.59 ± 5.36 cm³) and the left was (14.98 ± 10.77 cm³), values that are much higher than those found by Jasim and Al-Taei, (2013).²⁹ Jasim and Al-Tai (2013) justified the difference in results with the fact that dried African crania where used no bony septum was found within the sinuses. Also, difference in sample size contributed.²⁸

The frontal sinuses are located in the frontal bone above the eyes with each sinus opening into the middle meatus via the infundibulum. They are not obvious at birth and begin to develop during the second year of life. Radiographically frontal sinus is not evident before the age of five years. It is commonly acknowledged that the frontal sinus is completely developed by the age of 20 years and remains unchanged until further broadening of the chambers which occurs due to bone resorption during advanced age.³⁰ According to literature, it has been emphasized that frontal sinuses show significant differences in shape and capacity. Several studies show that the two frontal sinuses (right and left sinus) are rarely symmetrical. Due to its irregularities in shape and uniqueness to an individual, it is of most interest and significance in forensic identification.³¹ Belaldavar et al., (2016) conducted a study on an Indian population to assess frontal sinus as an aid for sexing and found that the mean values of the frontal sinus height, width and area are greater in males. Moreover, the right frontal sinus was larger than the left sinus in both male and female. The logistic regression analysis indicates that sex was accurately determined up to 64.6%.³² In another study, Uthman et al., (2010) used CT scan of frontal sinus on Iraqi population to assess sex and found an accuracy of 76.9% using discriminant functional analysis.

Nevertheless, when frontal sinus measurement was combined with skull measurement, an accuracy of 85.9% in sex determination was observed. Therefore, Uthman et al., (2010) concluded that frontal sinus can establish sexual dimorphism better but can accurately discriminate sex when combined with skull measurements. These findings are in agreement with other studies that indicate that the use of sinuses for forensic purpose alone may not be reliable as they are vulnerable to structural and developmental changes. Also, differences in radiographic techniques, such as distance, angle and orientation of the cranium can alter the image of the sinus, changing its anatomical characteristics. It is for this reason that this should be a supplementary method to reinforce findings from other methods.³⁰⁻³³

Soft tissue analysis

The scrutiny of soft tissue includes the study of lip prints (cheiloscropy) and study of palatal rugae patterns (rugoscopy).

Cheiloscropy

Cheiloscropy, derived from a Greek word “cheliōs” meaning “lips” and “skopein” meaning “see” is the term given to the study of lip prints. Two Japanese scientists, Y. Tsuchihashi and T. Suzuki in the period 1968 discovered that the patterns of lines on the red part of the human lip is unique to each person and can be useful for identification. Lip prints can be observed as early as 6th week of intrauterine life and remain unchanged for the rest of one’s life. During a crime scene investigation, lip prints can tie a subject to a specific location if found on cloths, glasses, cups or even cigarette butts.³⁴

These lip prints are classified by Suzuki and Tsuchihashi as follows:

- **Type I:** Clear- cut grooves running vertically across the lip
- **Type I’:** The grooves are straight but disappear half-way instead of covering the entire breadth of the lip
- **Type II:** The Grooves are branched
- **Type III:** The Grooves intersect
- **Type IV:** The Grooves are reticulate
- **Type V:** Undetermined³⁴

Vahanwala et al., (2005)³⁵ in their study reported that sex of the individual can be identified by lip prints dominance as follows:

- Type I, I’ pattern dominant: Female
- Type I and II patterns are dominant: Female
- Type III pattern dominant: Male
- Type IV patterns: Male
- Type V varied patterns: Male.

Many other studies used cheiloscropy to determine sex using Suzuki and Tsuchihashi’s classification and agree that the 10 mm wide area in the middle part of lower lip is the best- suited area of investigation.¹⁷³

Rugoscopy

Palatal Rugoscopy is the study of the pattern on the palatal rugae to identify an individual. Rugoscopy was initially suggested by a Spanish man Trobo Hermosa in 1932. Thomas and van Wyk (1988)³⁶ classified the palatal rugae pattern based on their length and shape. Based on length, it is classified as follows:

- Primary rugae (5–10 mm)
- Secondary rugae (3–5 mm)
- Fragmentary rugae (<3 mm).

Based on the shape it is classified as:

- **Straight:** Runs directly from the origin to termination
- **Curvy:** A simple crescent shape which was curved gently
- **Circular:** A definite continuous ring formation
- **Wavy:** Serpentine form

Literature reports several studies conducted on rugoscopy to analyze the patterns of the rugae. Subramanian and Jagannathan (2015)³⁷ carried out a study in an Indian population and reported no statistical difference in number of rugae in both male and female. Furthermore, fragmented rugae were found to be significantly increased in females as compared with males. There was also a gender difference in the length of the rugae with females having longer rugae, however, it was not statistically significant. These results are in agreement with Saraf et al., (2011) findings.³⁸

Molecular analysis

Highly degraded human remains presents a great challenge in the anthropological and forensic fields, hence hindering the smooth process of sexing. By tradition, it has been based on visual analysis of the sexual dimorphism present in the human bones. But, these kinds of methods are not suitable if the remains are incomplete or highly spoiled. Hence, much effort is pumped into research to establish alternative methods for sex determination and molecular biology is one of the methods that received immense attention. Due to the unique composition and structure of DNA molecule in bone and teeth, it is protected from environmental factors.³⁹ Various studies have been conducted to evaluate different dental tissues (pulp, dentin, and cement) for the presence of DNA useful for forensic analysis and often the conclusion is that the pulp consist of several cells rich in DNA and suitable for forensic investigations.⁴⁰ Most of the genomic DNA isolation methods use organic solvents such as phenol/chloroform, silica-binding extraction from powdered bone or teeth material. The extracted DNA from the teeth of an anonymous person can be matched with the ante mortem DNA samples for a positive identification. DNA stored in blood, hairbrush, clothes, cervical smear, or biopsy sample can provide a good source of ante mortem DNA.

Sex determination by molecular biology is ideal because variation in size and architecture of skeletal material has no influence on the biological molecules. Furthermore, foetal and juvenile remains gender can be determined. The method is not hindered by the quality of the sample and works perfectly well with low quantity. However, molecular methods for sexing also present some drawbacks with contamination being the greatest problem. Various environmental factors can induce molecular degradation and thus severely impair the process of obtaining DNA for forensic scrutiny. Furthermore, molecular methods can be costly and thus their use is often restricted to forensic material where other methods are not useful³⁹

Barr Bodies

In cases of an injury or murder from an assault where a single tooth is the only evidence left at the crime scene, the tooth can be used to determine the gender of its owner. Several studies have shown that when chromosomes are stained with quinacrine mustard, they fluoresce differentially along their length when viewed under ultraviolet light.⁴¹ Barr bodies (sex chromatin), are small well defined bodies found in nuclei of cells in females and are stained intensely by nuclear dyes. Murray Barr (1949)⁴² initially studied these structures, when he and coworkers analyzed nerve cells of cats in which they appreciated a high percentage of a dense mass of chromatin in cell nuclei of females, unlike in male. Those structures were then termed Barr bodies. The chromatin materials are representatives of one of the inactive X chromosome in each somatic cell in females that occurs during early embryonic development. Duffy and coworkers in a study carried out in 1991 examined human dehydrated pulps from extracted teeth to assess sex chromatin from fibroblasts in artificially mummified and heated pulp tissues and discovered that there is a prolonged sex chromatin stability.⁴¹ Similarly, several studies evaluated the in vitro effect of high temperatures on Barr chromatin in dental pulp for sex diagnosis. These studies indicated that Barr bodies are noticeable at temperatures up to 400 °C. At 600 °C, observation of the Barr bodies becomes difficult as the cells appear disorganized. At elevated temperature of 800 - 1000 °C it is impossible to view the Barr bodies as no viable tissue could be found. A conclusion was reached that sex of an individual exposed to elevated temperatures (up to 400 °C), such as fire and bombings, in which other methods cannot be used, can still be determined from teeth.⁴³ But, considering the fact that the studies are in vitro, where the teeth are directly exposed to fire, it may be arguable whether 400 °C is indeed the limit of observing the Barr bodies from the pulp. The experimental environment is not the same as the environment in vivo. The teeth are protected by soft tissues of the oral cavity and the pulp is further shielded by mineralized components of the tooth. Therefore, the temperature that goes directly to the tooth is much lower compared to the external medium that causes the fire or the explosion. Also, the time of exposure to fire of a body is variable. It is therefore necessary to set up experimental conditions that closely mimic the real situation, also, time of exposure should be investigated in future.

F bodies

The Y chromosome is unique in that it is only found in male. A fluorescent dye, quinacrine, binds strongly to the Y chromosome and a bright fluorescent spot (F body) is clearly seen under ultraviolet light. The motive behind the bright fluorescence of the Y chromosome is not entirely clear, however, Caspersson et al., (1970)⁴⁴ mentioned that alkylating agents such as quinacrine accumulate in DNA regions rich in guanine. The presence of F bodies infers that the DNA sample would most likely belong to a male, ruling out the female suspect. In the study by Whitaker,⁴⁵ assessment of gender was accurate in 30-100% of cases in tissues putrefying up to four weeks after extraction of the teeth, but, results indicate that accuracy was reduced in specimens left for 6-10 weeks. Nayar et al., (2014)⁴⁶ used fluorescent microscopy in their study to determine

sex from pulp tissue and pointed out that sex determination by fluorescent staining of the Y chromosome is a dependable technique. In many other related studies it is highlighted that post-mortem delicateness of the cells makes it difficult to separate cells from the hard pulpal tissue but not impossible. However, thin smears enable the clear visibility of the intact and observable cells.⁴³⁻⁴⁶ Das et al., (2004) studied the effect of temperature and humidity (in the mortuary) on sex chromatin of cadavers and reported that temperature did not have much effect on the number of F bodies detected, but, increase in humidity reduced the detectable F bodies as wet conditions accelerate the process of autolysis.⁴⁷

Enamel protein

In spite of the wide list of molecular methods recommended for sex assessment, amplification of the human amelogenin gene (AMEL) is often used. Amelogenin is a major matrix protein that is involved in the process of producing the enamel and is crucial for normal tooth development. The developing human enamel has approximately 30% protein, of which 90% are amelogenins.⁴⁸ Literature indicates that the person that initially sequenced the AMEL gene is Nakahori et al., (1991) and the gene is found on both Y and X chromosome. The unique organization and properties of the gene qualifies it to be an excellent tool for sex identification especially, from complicated forensic materials, such as highly fragmented, burnt, juvenile and foetal remains where sex cannot be estimated with traditional morphometric methods. There are arrangement and size differences between the male and female AMEL gene. According to Nakahori et al., (1991), AMEL X-allele has a size of 2872 base pair and is positioned on the Xp22.1–Xp22.3 area of X-chromosome, while the human AMEL Y-allele has a size of 3272 base pair and is positioned on the Yp11.2 section of Y-chromosome. Male individuals (XY) have two different AMEL alleles, one situated on chromosome X and the other on the Y chromosome, whereas, female (XX) have two matching AMEL alleles both situated on X chromosome. It is this difference that makes AMEL gene a powerful tool for sex identification.⁴⁹ Urbani et al. used the gene to determine sex and to assess the level of molecular degradation produced by varying high temperatures. This study was conducted to examine the remains of forensic victims of fire and to determine at what temperatures human teeth can still retain intact, useable DNA for identification.⁵⁰

II. Advanced Methods

Polymerase chain reaction

Polymerase chain reaction (PCR) is a revolutionary method established by Kary Mullis in the 1980s which has the ability to amplify trivial amounts of relatively short target sequences of DNA using sequence-specific oligonucleotide primers and thermostable Taq DNA polymerase. Teeth can endure high temperature and are used for personal identification in the forensic field. In the case of few teeth or lack of antemortem dental records, there is insufficient information for a positive identification of an individual. The dental pulp, being shielded by hard tissue, is well protected and is not influenced by temperature, unlike other structures of the oral cavity. Hence it is a major source for DNA used in PCR for amplification.⁵¹ Malaver and Yunis (2003) reported that pulp produced strongest PCR amplification signals while dentin and cementum signals were very similar to each other.⁵² Tsuchimochi et al., (2002) carried out a study to evaluate sex using DNA extracted from teeth by Chelex method and amplified specific male sequence with PCR and successfully determined sex.⁵¹ Sivagami et al. prepared DNA from teeth of Indians by ultra-sonication, followed by PCR amplification and they obtained 100% success in determining the sex of the individual.⁵³ Similarly, George et al., (2010) determined gender by PCR amplification of sex determining region “Y” gene (SRY gene) from removable partial denture contaminated with saliva and concluded that the dentures can be used as a source of forensic DNA. This, along with co-amplification of SRY gene and other routine sex typing markers will give definite gender identification.⁵⁴

Miscellaneous methods

Stenberg and Borrman in 1998 identified that labeling of dental prostheses with some information of the patient may be helpful in forensic investigations. The labeling can be done by inclusion system (using metal, nonmetal, micro label, and chips for labeling) and marking system (marking with spirit based pen or pencil). Considering the inert nature, toughness and capability to withstand high temperatures of the oral cavity, Borrman et al., (2013) advise lead paper to be used as denture markers. Many authors are in agreement that this labeled prosthesis can be used to identify a dead unknown body.⁵⁵

III. Conclusion

Forensic Odontologists play a vital role in the process of identification. In instances when only fragments of the body are recovered, the tooth is the chief tool that can be used to determine sex of an individual. Determination of sex is particularly essential as it can really rule out a certain percentage of

possibilities immediately. Dentition allows simple and easy methods for sex determination as well as the more advanced and sophisticated techniques. Depending on the circumstances presented at that time, the suitable and appropriate method should be used. It is therefore very important that a Forensic Odontologist is acquainted and familiar with most, if not all of the methods for sex determination by dentition. In addition, awareness of pelvic and cranial traits for sex determination by a Forensic Odontologist is worthwhile as such details can supplement and reinforce findings from dentition.

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