Review Article

Sex Identification in Forensic Odontology- A Review of Various Methodology

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ABSTRACT

Forensic odontology is the investigative part of dentistry that applies dental principles to legal issues that analyses dental evidence for human identification. Sex determination is a subdivision of forensic odontology, and it is very important, especially when information relating to the deceased is unavailable. The compilation and critical reading are necessary to understand the role of forensic odontology expert with regard to sex determination using dental records. This article reviews upon the various methods used in sex determination in forensic odontology.

Key Words: *Forensic odontology, investigations, sex determination*

Introduction

Forensic odontology is the application of dental principles to legal issues.

Dental identification involves either comparative method or postmortem dental profiling. The main advantage of dental evidence is that it can be preserved indefinitely after the death. The unique pattern of tooth enables the analysis of antemortem and postmortem dental variables. The main problem in dental identification is mostly related to acquiring interpreting antemortem records. Sometimes, because of the time duration, there will be variation while comparing these records. Various methods have been used for the identification of sex of the specimen. Sex determination becomes the first priority by a forensic investigator in the process of identification of bodies mutilated beyond recognition due to major mass disaster such as in the case of mishaps, chemical and nuclear bomb explosions, natural disasters crime investigations, and ethnic studies.[1] Determination of sex using skeletal remains presents a great problem to forensic experts, especially when only fragments of the body are recovered. Forensic dentists can assist other experts to determine sex of the remains using teeth and skull. Various features of teeth, such as morphology, crown size, and root lengths, are characteristic for male and female sexes. There are also differences in the skull patterns. These will help a forensic odontologist to identify the sex.

Sex Determination Analysis

Sex determination analysis can be done either by morphological analysis or by molecular analysis.

Morphological analysis can be done on hard tissues of oral and paraoral regions or soft tissue and other advanced methods.

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Morphological Analysis

Hard tissue analysis

Sex differences in tooth size

Teeth may be used for differentiating sex by measuring their mesiodistal and buccolingual dimensions.[2] This is of special importance in young individuals where skeletal secondary sexual characters have not yet developed.[3] Studies show significant differences between male and female permanent and deciduous tooth crown dimension. One has to be reminded that tooth size, or odontometrics, is under considerable influence of the environment. Such measurements are, therefore, population specific, and do not apply to the world at large. Among teeth, mandibular canines show the greatest dimensional difference with larger teeth in males than in females. Premolars, first and second molars, as well as maxillary incisors, are also known to have significant differences.

Sex determination using canine dimorphism

In the field of forensic odontology, permanent canine teeth and their arch width (distance between the canine tips) contribute to sex identification through dimorphism. The study of permanent mandibular and maxillary canine teeth offers certain advantages in that they are the least extracted teeth, are less affected by periodontal disease and the last teeth to be extracted in respect of age done by Bossert and Marks.[4] A study by Anderson and Thompson[5] showed that mandibular canine width and inter-canine distance was greater in males than in females and permitted a 59.7%-66.7% correct classification of sex. Garn *et al*.[6] studied sexual dimorphism by measuring the mesiodistal width of canine teeth in different

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ethnic groups. They concluded that the magnitude of canine teeth sexual dimorphism varies among different ethnic groups. Furthermore, the mandibular canine showed a greater degree of sexual dimorphism than the maxillary canine. Rao *et al*.[7] reported that the mesiodistal width of mandibular canines was significantly greater in males than in females. In another study of Rao *et al*.[8] 88% accuracy of sex identification was reported. Sherfudhin and *et al*.[9] investigated the occurrence of canine tooth dimorphism in Indian subjects and the use of two statistical methods of evaluation compared. Parameters considered were (i) the mesiodistal width of maxillary and mandibular canines, (ii) the maxillary canine arch width (inter-canine distance), and (iii) the mandibular canine arch width. The results indicated significant dimorphism of the maxillary and mandibular canine teeth. The sexual dimorphism specific to canines has been explained by Eimerl and DeVore[10] on the basis of their function which, from an evolutionary point of view, is different from other teeth. During the evolution of primates, there was a transfer of aggressive function on from the canines in apes to the fingers in man. Until this transfer was complete, survival of the species was dependent on the canines, especially those of males.

Mandibular inter-canine arch width

The cut-off point, or standard mandibular canine index (MCI) value, obtained by Rao *et al*.[7] was 0.274. If the MCI value of a skull specimen is less than or equal to the standard MCI, the individual is categorized as female; a value more than the standard MCI would group the person as male.

Dental index

In addition to absolute tooth size, tooth proportions have been suggested for differentiating the sexes. Aitchison[11] presented the "incisor index (Ii)," which is calculated by the formula,

Ii = [MDI2/MDI1] × 100,

Where MDI2 is the maximum mesiodistal diameter of the maxillary lateral incisor and MDI1 is the maximum mesiodistal diameter of the central incisor. This index is higher in males, confirming the suggestion of Schrantz and Bartha[12] that the lateral incisor is distinctly smaller than the central incisor in females. Another index, the "mandibular canine index" proposed by Rao *et al*.[7] and associates has given an accurate indication of sex in an Indian population. Using the mesiodistal (m-d) dimension of the mandibular canines, these researchers obtained the formula:

([mean m-d canine dimension + [mean m-d canine dimension in female + SD] in males − SD])/2.

Where SD: Standard deviation. m-d: Mesiodistal

The value obtained using this formula was 7.1, i.e., 7.1 mm is the maximum possible mesiodistal dimension of mandibular canines in females. The same dimension is greater in males. The success rate of determining sex using the above formula was close to 89%.

Root length and crown diameter

Using an optical scanner and radiogrammetric measurements on mandibular permanent teeth sex determination can be done with 80% accuracy by measuring root length and crown diameters.

Tooth morphology and sexing

In addition to the canines being the most sexually dimorphic teeth in terms of size, Scott and Turner 2nd[13] highlight that the "Distal Accessory Ridge," "a nonmetric feature on the canine" "is the most sexually dimorphic crown trait in the human dentition, with males showing significantly higher frequencies and more pronounced expression than females." Rao *et al*. and Rao *et al*.[7,8] have reported greater incidence of four cusps (absence of the distobuccal cusp or distal cusp) on the mandibular first molar in females (40.6%) compared to males (16.2%) in a south Indian population. They cite Anderson and Thompson[5] who opine that the reduction in the number of cusps is a reflection of an evolutionary trend toward overall reduction in the size of the lower face, with male apparently resisting this trend.

Orthometric method

Orthometric method involves morphology of skull and mandible with a constellation osix traits and frontal sinus dimensions. Neville *et al*[14] found sex could be predicted correctly in 96% of cases using different features of skull and mandible.

The constellation of six traits are mastoid, supraorbital ridge, size and architecture of skull, zygomatic extensions, nasal aperture, and mandible gonial angle and it was said that the determination of sex using only these six traits shows the accuracy of 94%.

Frontal sinus dimensions

Sinuses are mucosa-lined air spaces within the bones of the face and skull. Frontal sinuses are situated between the internal and external laminae of the frontal bone. Frontal sinuses are absent at birth and fully developed around 8 years and reaches full size after puberty. Frontal sinuses are important parameters in the determination of sex as it presents a distinctive differences in shape, measurements, and symmetry. Uthman *et al*.[15] in their study of evolution of frontal sinuses and frontal measurements using spiral computed tomography scanning of ninety patients concluded that frontal sinus measurements are valuable aid in differentiating sex and stated that, including skull measurements along with frontal sinus measurements improved the accuracy. Belaldavar *et al*.[16] showed a greater mean values of frontal sinus height, width, and area in male compared to female.

Soft tissue analysis

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

Cheiloscopy

The word chelios comes from the Greek word meaning lip. The study of lip prints is called cheiloscopy. Lip prints can be identified even at 6th week of intrauterine life. These prints do not change after that. These lip prints are classified by Suzuki and Tsuchihashi[17] 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: Undetermined.

Vahanwala *et al*.[18] in their study concluded that sex of the individual can be identified by lip prints 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.

Similar findings were reported by many other authors.

Therefore, lip prints are unique patterns on lip which help in identification of a person. The 10 mm wide area in the middle part of lower lip is used as the best-suited area of study.

Rugoscopy

Trobo Hermosa, a Spanish investigator in 1932 first proposed on palatal rugoscopy. Palatal rugoscopy or rugoscopy is the study of the pattern on the palatal rugae to identify a person. Due to its internal position, stability, perennity that is, it persists throughout life, it is selected in forensic for human identification. Thomas and van Wyk[19] classified the palatal rugae pattern based on their number, 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.

Various studies have compared the rugae pattern in male and female. A study on Japanese population concluded that female had fewer rugae than male. Shetty *et al*.[20] compared the rugae pattern between Indian and Tibetan population. The result of their study showed that Indian male possessed more primary palatal rugae on the left side when compared to female. And also more curved rugae was observed in Indian male compared to Tibetan male and more wavy rugae was observed in Tibetan female when compared to Indian females. Study results of Bharath *et al*.[21] among males and females showed a specific pattern of palatal rugae pattern among both sex of coastal Andhra population.

Molecular analysis

Since morphological patterns vary with time and external factors, the best-suited method in the identification of gender is by molecular analysis of DNA. The extracted DNA from the teeth of an unidentified person can be compared with the antemortem DNA samples. DNA stored in blood, hairbrush, clothes, cervical smear, or biopsy sample can provide a good source of antemortem DNA.

The different types of DNA are nuclear DNA and mitochondrial DNA. Extraction of DNA can be done either cryogenic grinding which involves cooling the whole tooth to extreme low temperature using liquid nitrogen, and grind the tooth to extract the DNA. The lesser destructive method for DNA isolation involves opening of root canals and scraping the pulp area with a notched medical needles. The extracted DNA can be analyzed by various methods such as restriction fragment length polymorphism, polymerase chain reaction (PCR), and microarrays.

Sex determination using barr bodies

Sex can also be determined by the study of X and Y chromosomes in the cells which are not undergoing active division. Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp. After death it persists for variable periods depending on the humidity and temperature in which tissue has remained. Deeply stained X chromatin and intra-nuclear structure in females is also known as Barr body as it was first discovered by Barr and Bertam.[22] It is present as a mass usually lying against the nuclear membrane in the females. Whittaker *et al*.[23] determined sex from necrotic pulp tissue stained by quinacrine mustard using fluorescent Y chromosome test for maleness and claimed that up to 5 weeks sex determination can be done with high degree of accuracy. It was found that in cases after fires, high impact crashes and explosions fragmentation and thermal trauma renders other methods impossible to determine sex of the remains except the above said method from pulp. Pulp tissue cells become embedded firmly into the dried fibrosis matrix. Duffy *et al*.[24] have shown that Barr bodies and F-bodies of Y chromosomes are preserved in dehydrated pulp tissues up to 1 year, and pulp tissues retain sex diagnostic characteristics when heated up to 100C for 1 h. As Barr bodies are seen with the nucleus, they can be visualized by various special staining procedures like papanicolaou stain. Negative results can be attained under certain pathological conditions as they can be associated with variations in size and shape of Barr bodies.

F-bodies

Y chromosome contains F-bodies. These F-bodies can be used to identify sex. Various studies have been undertaken to identify F-bodies from pulpal tissue. Caspersson *et al*.[25] suggested that F-bodies can be applicable in forensic for sex determination. Dried blood stains, saliva, hair, and extracted dental pulp can serve as a sample for the test. Seno and Ishizu[26] carried out the detection of Y chromosome in the nuclei of dental pulp. Their study result was that over 30% of the male pulpal tissue showed positivity for F-bodies. F-bodies could be examined even in teeth as old as 5 months after extraction. Nayar *et al*.[27] in their study of pulp tissue in sex determination using fluorescent microscopy concluded that sex determination by fluorescent staining of the Y chromosome is a reliable technique in teeth with healthy pulps or caries within enamel or up to half the way of dentin. Teeth with caries involving pulp cannot used for sex determination.

Sex determining region "Y" gene

The abbreviation of SRY is the sex determining region "Y" gene. These gene codes for the sex-determining region Y protein, which is responsible for further development as male. Females have 2X chromosomes (46XX), and males have 1X and 1Y chromosome (46XY). SRY is located on the short (p) arm of the Y chromosomes at the position 11.3. Therefore, SRY gene can be used as a sex-typing marker in forensic samples. Many studies have shown the amplified SRY gene in various samples to determine sex. False positive results can be attained in certain syndromes, maternal-fetal microchimerism, and dissimilar sex between donor and recipient during transplantation (chimerism). George *et al*.[28] identified gender by amplification of SRY gene using real-time PCR from isolated epithelial cells of the removable partial denture. They concluded that saliva-stained acrylic dentures can act as a source of forensic DNA and co-amplification of SRY gene with other routine sex-typing markers will give unambiguous gender identification. Reddy *et al*.[29] studied the epithelial cells adherent to toothbrush as a source of DNA for sex determination using real-time PCR. All male sample in their study showed positive results and out of 15 female samples four were wrongly identified as males.

Sex determination from the enamel protein - amelogenin gene

Amelogenin (AMEL) is a major matrix proteins found in the human enamel involving Amelogenesis. Developing human enamel has about 30% protein, 90% of which are AMELs. AMEL gene is involved in the formation of AMEL. AMEL X gene is present in 106 bps and AMEL Y is present in 112 bps of the DNA. It has a different signature (or size and pattern of the nucleotide sequence) in male and female enamel.

The AMEL gene that encodes for female AMEL is located on the X chromosome and AMEL gene that encodes for male AMEL is located on the Y chromosome. The female has two identical AMEL genes or alleles, whereas the male has two different AMEL genes. This can be used to determine the sex of the remains with very small samples of DNA.[14]

Advanced methods

Sex determination using polymerase chain reaction

PCR is a method of amplifying small quantities of relatively short target sequences of DNA using sequence-specific oligonucleotide primers and thermostable Taq DNA polymerase. The teeth can withstand high temperature and are used for personal identification in forensic medicine. In the case of few teeth or missing dental records, there is not enough information to identify the person. The dental pulp enclosed by the hard tissue is not influenced by temperature, unlike the buccal mucous membrane, saliva, and calculus. A procedure utilizing Chele × 100, chelating resin, was adapted to extract DNA from dental pulp. In a study by Tsuchimochi *et al*.[30] used Chelex method to extract DNA from the dental pulp and amplified it with PCR and typing at Y-chromosomal loci to determine the effects of temperature on the sex determination of the teeth. Hanaoka and Minaguchi[31] conducted a study to determine sex from blood and teeth by PCR amplification of the alphoid satellite family using amplification of X (131 bp) and Y (172 bp) specific sequences in males and Y-specific sequences in females. It was showed to be a useful method in determining the sex of an individual. Sivagami *et al*.[32] prepared DNA from teeth by ultrasonication, and subsequent PCR amplification, they obtained 100% success in determining the sex of the individual.

Conclusion

Sex determination in forensic odontology can be done by either on morphological analysis or molecular analysis. Morphological variations linking to sex can be done on either hard or soft tissue. Analysis of hard tissue includes variation in morphology of tooth dimension (odontometric method) and variations in morphological traits of the skull (orthometric method) and few miscellaneous methods like denture labels. Cheiloscopy and rugoscopy come under soft tissue analysis. Molecular analysis involves the study of DNA from extracted pulp, cartilage, hair, skin. Buccal mucosa, epithelium attached to denture and toothbrush. Barr bodies, F-bodies, SRY gene, AMEL gene can be studied to determine sex from these samples. A thorough knowledge and usage of the appropriate evidence from forensic scene enables proper identification of the individual.

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Conflicts of interest

There are no conflicts of interest.

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