



Review Article

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Gender Determination in Unidentified Cadavers from Skull and Teeth Measurements

Konstantinos Vlasidis^{1*} and Maria Koutsamani²

¹Dentist Director, University Hospital of Crete, Greece

²Dentist Director, Kastelli Health Center, Heraklion

*Corresponding author: Konstantinos Vlasidis, Dental Clinic, University Hospital of Crete Heraklion, Greece.

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Abstract

Sex determination is a highly important procedure for a plethora of sciences, including the modern science of Forensic Dentistry. The role of the specialized, experienced and well-trained dentist is deemed necessary especially in cases of criminal acts or mass disasters, where corpses can be identified, as well as skeletal or dental remains of unknown deceased individuals that need immediate identification or finding data related to gender, race, age, health and others that can be used by local authorities for a successful identification. For this reason, various morphometric and morphological methods are used mainly on skull and teeth along with various methods of biochemical analysis of genetic material and Barr bodies that can be extracted from them. The present structures are selected because they carry certain characteristics of sexual dimorphism capable of facilitating any differentiations between the two biological sexes. In particular, this essay describes the measurements of dimensions (width, length, height and distances), the shape, size and enamel of the teeth, but also of other cranial structures, such as the jaws, the occipital, frontal, temporal and zygomatic bones and the palate. The ultimate goal is a valid sex determination and classification with as high an accuracy index as possible with the contribution of special tools, such as calipers, and advanced digital technology.

Keywords: Gender determination, Forensic dentistry, Skull measurements, Teeth measurements

Introduction

Sex determination from osteological and dental records has long been an interdisciplinary field of dentistry as well as of forensics and anthropology [1,2]. In terms of "Forensic Dentistry", in particular, highly trained dentists with expertise, experience, and regular training in the field of forensics are recruited to assist in the process of identifying victims of criminal acts, mass environmental disasters, traffic accidents and others [3,4]. Thanks to the development of technology, there is a great advance in sex determination, where if the entire skeleton is available, the accuracy can reach 98%, with the pelvic bones alone providing about 95% accuracy [5]. However, in cases of finding skeletal remains, parts of the cranial cavity or isolated teeth, then gender identification is considered an interesting and at the same time complex process,

often challenging the specialist dentist, who is called upon to apply various morphological and morphometric methods of skull and teeth measurement, but also biochemical methods for analyzing genetic material and Barr bodies (sex chromatin) for the present purpose [1,6,7].

Gender Determination and Types of Measurement Methods

The determination of biological sex is based on the recording and study of all those gender/sexual dimorphic characteristics that exist in a person's physiology and classify them as male and female. It is emphasized that this paper mentions biological gender and not social gender, which is a consequence of the demands that each society creates and places on its members, is not explained on the



basis of biology or genetics, while it refers to those particular traits and behavioral patterns that describe the concepts of “masculinity” and “femininity”. A number of studies have been conducted to identify cranial and dental differences in males and females for sex determination in unidentified cadavers [1,8-10].

As described below, the determination of gender in cadavers or remains of skulls and teeth can be achieved through specific measurement methods. The first concerns the non-metric morphological assessment of traits that show significant sexual dimorphism and is based on the subjective evaluation and experience of the researcher [6,11]. The second is the morphometric assessment based on the precise determination of anatomical cranial and dental landmarks that requires the use of special tools, such as calipers, and digital software [8,12]. The third, finally, is related to biochemical analysis, in the form of genetic material analysis or Barr bodies (sex chromatin) analysis through microscopy. Of course, the fact is underlined that due to the existence of variations between different population groups in measurements mainly in the skull, it is recommended that the analysis of the measurements not be generalized, in order to avoid unsafe and unreliable conclusions [1,7].

However, it is stated that with the help of computers, the process of conducting especially morphometric measurements and obtaining results has been accelerated and is judged to be more objective. Also, through 3D image analysis, dentists are given the opportunity to perform a rapid and automatic measurement of the entire external surface of the skull, instead of measurements based on certain characteristics only, such as its distances and angles [6,13]. Finally, the possibility of storing all these extracted data in digital form offers the possibility of multiple analyzes over time, which significantly contributes to gender estimation and the identification of skeletal remains [6].

Morphological and Morphometric Teeth Measurements

First of all, it is emphasized that the method of identifying a deceased person based on data from his dental image is considered essential, as teeth show exceptional resistance in conditions where the body is exposed to environmental changes, such as humidity and high temperatures, or in cases of trauma and other injuries, even after death [9,14]. The forensic dentists are called upon to record and analyze the post-mortem evidence they will extract from the identified teeth, but also to compare them with possible ante-mortem records, to facilitate the process of determining sex, race, age, dietary habits, possible prosthetic work, and other unique distinguishing features, in order to achieve positive identification [2]. Especially for gender determination, dental markers such as incisors, mandibular canines and crowns can be used, capable of revealing sexual dimorphism in the teeth [1,8-10].

For example, research by Rani (2017) involving dental measurements of 180 patients aged 18-25 years from the Nalgonda region of India has concluded that maxillary and mandibular canine proximal-distal width, mandibular left canine index, right incisor index upper jaw, but also the width of the meso-premolar and

meso-molars show great sexual dimorphism, as they appear much larger in men than in women [10]. In the present study, the result extraction was based on the use of the mathematical equation of Garn, et al. (1967) in finding sex differences in tooth size and shape. Briefly, a value with a positive sign means that male teeth are larger than female teeth, while a value with a negative sign indicates exactly the opposite. However, if the value is close to zero, it shows that the levels of sexual dimorphism are not high, so it is not considered possible to detect different dental characteristics between the two sexes [15].

The findings of Rani (2017) are confirmed by *Prasann, et al.* (2021), who performed measurements on teeth of 1200 students aged 18-25 years of South Indian origin using a vernier caliper and concluded that the maximum proximal-distal diameter of all mandibular incisors is greater in male subjects than in females, while the maxillary incisor index appears larger in females than in males. Another characteristic of sexual dimorphism is found in the length of the root and the diameter of the crown of the teeth [9,10]. In particular, using an optical scanner and radiological measurements on mandibular permanent teeth, gender can be determined with an accuracy of 80% by measuring root and crown diameters [6,16].

In addition, permanent canines and the width of the dental arch contribute to gender identification through magnetic imaging, namely the SWIFT (“Sweep Imaging with Fourier Transformation”) method, but also through the measurement of linear dimensions, such as proximal-distal and buccal-labial width. Indeed, the proximal-distal width of the maxillary and, especially, mandibular canines appears significantly greater in men than in women in various ethnic groups, with the overall sex determination rate reaching 79% of the cases in the examined samples [17]. Furthermore, in terms of morphology, the canine crest is another non-metric feature of sexual dimorphism of the human dentition, as it appears to protrude more in males than in females [6]. Finally, studies have shown that the increase in mass body size corresponds to an isometric increase in tooth volume, with males having larger teeth than females. Despite this, divergent amounts of enamel are observed between the two sexes, since in women, as seen by CT scan, it is relatively thicker, which is another distinct feature of sexual dimorphism [18,19].

Morphological and Morphometric Skull Measurements

Dimorphic features in sex determination are found in almost all bones, such as the ulna, metacarpals, metatarsals, pelvis and skull, with the latter showing an extremely high accuracy reaching 94% [12]. Anatomically, a multitude of cranial structures are used to determine gender, including the frontal, temporal, occipital, and zygomatic bones, the frontal sinus osteoma, and others. Indeed, related research by Amores-Ampuero (2017), who studied the skulls of 109 corpses (53 men and 56 women) of known sex, age, and cause of death from the San Jose cemetery, Granada, Spain, applying linear discriminant analysis in six specific dimensions, concluded that all values, particularly the length of the occipital condyle and the

width of the occipital foramen, were significantly higher in males than in females with a percentile rank of 75.7% (77.8% for males and 73.7% for women) [7,13,20].

Another related study that also aims to provide accuracy in gender estimation is that of Casado (2017), who, by the use of calipers, performed three-dimensional measurements of coordinates of six cranial features, including the supraorbital crest, the glabella, the external occipital protuberance, of the nuchal ligament and its lamellae, as well as the mastoid and styloid processes. The sample examined consisted of 158 skulls of Caucasian and African American men and women from New Mexico, USA, and the research showed that based on the present measurements, males were correctly classified by 69.9%, while females by 74.7% [13].

In support of the aforementioned, emphasis can be placed on frontal sinus dimensions as another feature of sexual dimorphism, showing variations in both shape and measurements and symmetry between the two sexes [7]. Specifically, *Uthman, et al.* (2010) in their study of frontal sinus development, conducted analogous measurements using computed tomography in 90 patients (45 women and 45 men) aged 20-49 years from Baghdad city, Iraq. These included the width, height, and anteroposterior length of the frontal sinus, the distance between the highest points of the two sinuses, but also that between the highest points of each sinus to the maximum lateral border along with measurements of maximum length, width, and anterior parietal skull height. The research concluded that measurements of the frontal sinus in combination with those of other skull structures can provide accurate sex determination results [21].

Belaldavar, et al., (2014) confirm and strengthen the aforementioned findings with a study conducted on 300 individuals of Indian origin (150 females and 150 males) aged 25-30 years, emphasizing the particular importance of frontal sinus and skull measurements collectively through digital radiographs, and showing a greater mean height value, frontal sinus width, and surface area in men compared to women [11].

A reliable method for gender determination is the analysis of linear measurements of the bones of the palate and the base of the skull. Indeed, in a study by *Lima, et al.* (2012) on one hundred skulls (50 male and 50 female) of deceased aged 22-55 years from the Sao Gondzalo cemetery of Cuiabá, Brazil, it was shown through a digital caliper and statistical analysis that the distances between the anterior part of the palatine foramen, the right and the left greater palatine foramen, and the base show sexual dimorphism. The skulls were correctly classified with a reliability rate of 63% for males and 65% for females, which shows that even the palatal bones show differences between the two sexes and, by extension, should be taken seriously [22]. In any case, however, more research among different population groups in both skull and teeth should be conducted to yield a greater index of precision and reliability [8,20].

Biochemical Methods

Teeth, and particularly canines and molars, are a rich source of genetic material and Barr bodies, the extraction of which, espe-

cially from enamel, dentin, cementum and pulp, can be compared with ante-mortem samples for a successful cadaveric identification or to determine its sex [7,23,24]. In particular, the dental pulp contains fibroblasts, odontoblasts, endothelial cells, peripheral nerves, undifferentiated mesenchymal cells and nucleated red blood cells, which are in the crown and root pulp, and which can provide a large amount of genetic material [23]. Biochemical methods of measurement that can be applied for the purpose of sex determination are based on the analysis of genetic material and Barr bodies from the pulp or from the hard tissue of the teeth [1,7]. It should be emphasized that as a procedure, the in vitro Polymerase Chain Reaction (PCR) technique used among others to isolate specific sequence of genetic material is considered more accurate and time-consuming to obtain results, while Barr bodies analysis is faster and requires less equipment [23].

Barr bodies (otherwise known as sex chromatin) are condensed, inactivated X chromosomes, which are found exclusively in female mammals and are found in various samples of biological material, such as hair, oral cavity cells, and blood [7,25]. Women have two X chromosomes, one of which is inactivated, and they carry such a body in their cells, while men, having only one X chromosome, do not have it [25]. It is considered imperative to determine both the presence or absence of X and Y chromosomes in the dental pulp, because due to temperature and humidity fluctuations, this tissue rapidly undergoes decay, which in turn gives equivocal or negative results in terms of presence of sex chromatin [1,24]. However, gender determination from the pulp is possible up to 7 weeks after death, which allows for safe and valid results [25].

A useful method of analysis and measurement applied to determine the gender of an individual is the use of PCR biochemical analysis on the amelogenin gene (AMEL) from the genetic material found in the dental pulp [23] (*Nagare, et al.*, 2019). Amelogenin is produced during the early stages of tooth formation and carries two types of genes, one on the X chromosome and the other on the Y chromosome [7,23]. However, there have been discrepancies in sex determination based on this gene, mainly due to "deletion" of the X and Y chromosomes in various populations, such as Asians and Indians, but also due to mutations [1]. The presence of PCR inhibitors and mixed genetic material, "degeneracy" of genetic material samples and others can also lead to inaccurate results obtained from the amelogenin assay, and therefore other alternative techniques and markers have been proposed for a more reliable gender determination, such as biochemical analysis of SRY, DYZ1 genes and Next Generation Sequencing (NGS).

Other methods of biochemical analysis and measurement include the use of fluorescence microscopy. In more detail, the dental pulp cells are stained with quinacrine hydrochloride and observed under UV light with a special microscope. Sex is determined by identifying the strong fluorescence of the Y chromosome in the dental pulp versus other chromosomes [14,19]. The reason for the bright fluorescence of the Y chromosome is not entirely clear [1]. This technique has been applied in forensic science to estimate gender from dried blood spots, saliva, and hair since the 1970s [16].

This economical technique is distinguished by ease of handling, but also by reliability in terms of gender determination valid up to one month after someone's death [1,14,19].

Conclusions

In conclusion, as demonstrated on the basis of relevant literature in this paper, the determination of biological sex in cadavers of unknown elements, as well as in skeletal or dental remains, is a special field that concerns a multitude of sciences, including forensic dentistry. Indeed, the work of the specialist dentist is considered quite demanding, as they are called upon to apply different assessment methods, both morphological and morphometric in nature, but also methods of biochemical analysis of genetic material and/or Barr bodies for accurate gender assessment. Special mention is made of the value of recording and studying measurements from various structures of the skull, such as the palate, frontal, temporal, occipital and zygomatic bone, the frontal sinus osteoma, the upper and lower jaw, as well as the cervical ligament, but also from the teeth, especially canines, incisors, premolars and molars, and their other dimensions (length, width, height and distances), always combined with analysis of extracted genetic material and sex chromatin, and other biochemical methods. These characteristics, according to various studies, are distinguished by sexual dimorphism and, by extension, present significant differences between the two genders.

The development of technology through 3D and other X-ray imaging and advanced digital software has facilitated the extraction of more accurate and objective results. Of course, even so, these assessment methods and techniques need further research and practical application in deceased of different population groups to confirm and further strengthen the reliability of the findings and correct gender classification. However, the specialist forensic dentist can contribute positively to the successful determination of the gender of skeletal remains, drawing up their profile, the comparison of post-mortem with ante-mortem data, if these are available and a detailed record is kept, but also to the subsequent effective identification of the deceased, in collaboration, of course, with the competent authorities.

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Conflict of Interest

None.

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