Gender Determination from Dental Pulp Tissue

Shilpi Sharma[1], Maria Priscilla David[2], Indira A.P.[3]

ABSTRACT

Establishment of a person's individuality is important for legal as well as humanitarian purpose and gender determination is an essential step in identifying an individual. The identification of a person is required when the body is disfigured or mutilated beyond recognition as a result of barbaric crimes, workplace and motor vehicle accidents, aviation and navy disasters, wars, fire, flood, manmade and natural mass disasters and casualties, and in circumstances where the body is in an unrecognizable, decomposed state.In teeth, DNA is found in the pulp tissue, dentin, cementum, periodontal ligament and alveolar bone. Teeth are one of the most robust structures of the human body and offer high impedance to the immediate and cumulative effects of adverse environmental conditions.Teeth can survive long after soft and skeletal tissues have been destroyed and since the pulp is enclosed in hard tissue structures, it becomes a reliable means for determination of sex.

Keywords: Gender Determination, Dental pulp, X Chromatin, Y Chromatin, DNA

INTRODUCTION

The main attributes of biological identity are sex, age, stature and ethnic background of the individual which are also called the 'Big four' in forensic context.[1] Sexing is the first step in the completion of the biological profile. Determination of sex of human remains is frequently immediate even in severely decomposed cadavers.[2] Numerous studies already exist on gender determination from primary anatomical parts such as the pelvis, skull bone, mandible, clavicle, femur and many other parts of the human skeleton which show gender difference. The pelvic bone is the best skeletal part to accomplish a reliable sexual diagnosis because it has long been recognized as the most dimorphic bone, particularly in adult individuals.[3]

The tooth is the most valuable source to extract DNA since it is a sealed box preserving DNA from extreme environmental conditions, except its apical entrance.Soft tissue within coronal and radicular pulp chamber consists of odontoblasts, fibroblasts, endothelial cells, peripheral nerve, undifferentiated mesenchymal cells and nucleated components of blood which are rich sources of DNA.[4] Pulp tissue is the most widely used option, since it is normally abundant and is less vulnerable to contamination by non-human DNA.[5]

METHODS OF RETRIEVAL OF THE PULP TISSUE

Sampling of pulp tissue is done in three ways: crushing, vertical or horizontal splitting, and by endodontic access.[6]

Crushing entire tooth

Extracting dental DNA for identification purposes is usually performed after crushing the tooth.The main disadvantage of this method is that the tooth is completely destroyed so further radiographic, anatomical or biochemical studies are no longer possible.[7] Moreover, the crushed sample has to undergo multiple cycles of decalcification and purification, and usable quantity of DNA may not be obtained.[6] In addition, this approach ignores the specific locations of dental DNA and may increase the likelihood of contamination by bacterial DNA endonucleases and potential polymerase chain reaction (PCR) inhibitors found on the exposed surfaces of the tooth.[8]

Conventional endodontic access

The other method is preparing a conventional access cavity on the tooth and excavating the pulp using a suitable hand instrument.[6] The technical difficulty of this approach depends on the pulp chamber morphology and size of the access opening. The greatest disadvantages are the disruption of the occlusal surface and restorations, as well as assuring that all portions of the chamber are completely debrided.[8]

Vertical split

A section along the vertical axis of the tooth allows convenient access to the entire length of the pulp chamber.[8] This method provides for easy excavation of the entire pulpal tissue with minimal chance of contamination.[6] Unfortunately, the eccentric nature of tooth roots in the vertical plane makes it nearly impossible to achieve longitudinal access to the entire pulp chamber in most multirooted teeth.[8]

The carborundum discs are used to split the tooth from the incisal edge, with frequent washing with distilled water. When the pulp cavity is reached, the tooth may be split by a chisel to avoid heat and mechanical damage to the pulp tissue. The pulp is then excavated and transferred to vials.[6]

Horizontal spitting

Horizontal splitting is essentially the same procedure and is done when the crown structure is to be preserved.[6] A horizontal section through the cervical root, subjacent to cementoenamel junction avoids most restorations and provides the operator access to both the radicular and coronal pulp chambers. This unhindered approach to root canals is an improvement over the conventional endodontic access.[8]

GENDER DETERMINATION FROMIDENTIFICATION OF X CHROMATIN

A sex difference in mammalian interphase cells was first described by Barr and Bertram (1949), who discovered a darkly staining body which was present in the nuclei of nerve cells from female cats and absent from the cells of male cats. The body was intimately associated with the nucleolus and was originally

[1]Post Graduate Student, [2]Professor and Head of Department, [3]Professor, M.R. Ambedkar Dental College and Hospital, Bangalore, India

Corresponding author: Dr. Shilpi Sharma. M.R. Ambedkar Dental College and Hospital. 1/36, Cline Road, Cooke Town. Bangalore – 560005, India

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called 'nucleolar satellite'.[9] Sex chromatin is characteristic of females (found in about 30-40% of female cells).[10] A Barr body is a small well-defined body which stains intensely with nuclear dyes. It is present in a large proportion of nuclei of female origin and absent in male nuclei.[11] Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp.[12] Klinger (I957) found that in amnion and connective tissue, 61.8% of the Barr bodies were at the nuclear periphery, 23.2% were lying against a nucleolus, and 9.2% were apparently free in the cytoplasm (although it cannot be excluded that a Barr body, which is seen in the centre of a nuclear profile is not, in fact, lying against the nuclear membrane in the three-dimensional nucleus).[11]

Stains for X chromatin

Barr body can be observed with most of the nuclear stains such as hematoxylin and eosin (H and E), thionine, Papanicolaou, Feulgen, cresyl-violet, giemsa, aceto-orcein, and under fluorescence such as acridine orange. Kaveri Surya Khanna conducted a study to demarcate human dental pulp as an important identification tool of sex in forensic odontology (FO) and to evaluate the time period till which sex can be determined from pulp tissue using three stains H and E, Feulgen, and acridine - orange under fluorescence so as. 90 pulp samples (45 males and 45 females) were subjected to Barr body analysis for determination of sex using light and fluorescent microscopy. Barr bodies were best identified under H and E stain at a time period of 15 days (51.4% cells positive) followed by Feulgen (37.33% cells) and least under AO (23.2% cells).The study of Barr bodies' analyses has an advantage over other techniques due to simple staining techniques and its less time consumption.[13]

Limitations of this method are because of the alterations at the chromosomal level, patients with abnormalities can yield false positive or false negative results. Sauzo et al in their study found that in control men, no Barr bodies were reported, and in 46XX women, one Barr body in cell nuclei was observed. However in men and women with aneuploidy, 47XXY men also have a Barr body, and 47XXX women have two.[14]

GENDER DETERMINATION FROM IDENTIFICATION OF Y CHROMATIN

In 1968, Caspersson et al reported that metaphase chromosomes exhibited various fluorescence patterns after staining with fluorescent dyes, and it was suggested that the technique might provide a useful tool for chromosome mapping.[15] It has been reported that the distal half of the long arm of the human Y chromosome shows a differential affinity for fluorescent acridine derivatives (Zech, 1969; Pearson, Bobrow and Vosa, 1970).[16] This region of the Y chromosome remains heteropycnotic in the interphasic nuclei, it is being evident as a strongly fluorescent body, named Q body, F body or Y chromatin (Beatty 1975, Hegde et al. 1978).[17] By the use of this staining reaction, the Y chromosome can be detected in interphase nuclei of lymphocytes, cultured skin fibroblasts and buccal mucosal cells. This technique has also been used to demonstrate that the X chromosome pairs with the short arm of the Y in first meiotic prophase (Pearson and Bobrow).[16] The F- body is typically described as a single fluorescent spot, approximately 0.25 um in diameter and of variable length, located halfway between the periphery and the centre of the nucleus.[18]

Stains for Y- chromatin

Quinacrine mustard (2-methoxy-6chloro-9,4–bis(2-chloroethyl) amino-1-methylbutylaminoacridinedihydrochloride) stains with particularly strong fluorescence. Quinacrine dihydrochloride, usually only referred to as quinacrine (2-methoxy-6-chloro-9,4 bis (2-ethyl)amino-1- methylbutylaminoacridinedihydrochloride, "atebrin", " mepacrin"), gives also an excellent differential staining, though a little weaker than quinacrine mustard and which perhaps fades more rapidly on exposure to UV- light. All quinacrine derivatives depend for their staining action on the intercalation of the acridine nucleus in the DNA molecule and on the ionic binding of the side chain to the phosphate groups of DNA. Weisblum and Haseth (1972) showed that the fluorescence of quinacrine is enhanced by AT- rich DNA and decreased by GC- rich DNA.Quinacrine mustard is especially recommended for quantitative studies. For qualitative investigation involving the differentiation of chromosomes and the visualization of the Y- chromatin, quinacrine dihydrochloride is very satisfactory.[19]

DAPI/Distamycin A (4, 6-Diamidino-2-phenylindole) is a fluorescent dye with DNA affinity for A+T- specific binding. When chromosomes are stained with this compound a banding pattern similar to Q-banding is seen, although contrast is poor. DAPI is widely used as a counterstain in FISH studies. Distamycin A also has an affinity for A+ T specific DNA but is non- fluorescent. The heterochromatic regions of chromosomes 1, 9; 16, the distal arm of the Y- chromosome and the proximal short arm of chromosome 15 stain brightly while all other regions appear dull.[20]

Veeraghavan et al conducted a study on extracted periodontally compromised teeth which were kept at room temperature without any preservation and inferred that Y body was reliable for gender determination upto 1 month and gender can be determined with reduced efficiency upto 5 months.[21]

The reason for the traditional view of the human Y chromosome as an all-powerful entity is the dominant role it plays in sex determination. This is evident from the sex of patients with abnormal numbers of sex chromosomes. Males with Klinefelter syndrome (XXY) have two X chromosomes, like a female, plus a Y chromosome, like a male, whereas females with Turner syndrome (XO) have a single X chromosome and no Y chromosome. Even patients with multiple X chromosomes (up to five) are male, if they possess a Y chromosome, and female, if they lack a Y chromosome.[22] Unlike FB, which can be easily identified by their fluorescence, identification and counting of Barr body seems to be difficult.[23]

There are some limitations of this method. There can be masking of the fluorescent Y chromosomes by fluorescent debris impeding the examination of intact nuclei which can be prevented by smearing the pulpal cells homogenously and thinly over the slides. Further, the bacteria, dead cells, and putrefied cellular debris may give false positive fluorescence particularly in case of female cells. Moreover, the procedure for quinacrine dihydrochloride staining is very technique sensitive, with each step right from the separation of pulp from the dental hard tissue until the counting of the bright fluorescent spots attached to the nucleus.[23]

GENES IN GENDER DETERMINATION

SRY GENE

The SRY gene marker (sex determining region Y) is a sex determining gene on the Y chromosome in the therians (placental mammals and marsupials) and this gene marker is considered as a signature gene to differentiate the male from female sex chromosomes.[24] SRY is located on the short (p) arm of the Y chromosomes at the position 11.3, more accurately, from base pair 2,786,854 to base pair 2,787,740. False positive results can be attained in certain syndromes, maternal – fetal microchimerism and dissimilar sex between donor and recipient during transplantation (chimerism).[25]

Amelogenin or AMEL is a major matrix protein found in human enamel. It has a different signature (size and pattern of nucleotide sequence) in male and female enamel. The AMEL gene that encodes for female amelogenin is located on X Chromosome and the AMEL gene that encodes for male amelogenin is located on Y Chromosome. The female has two identical AMEL genes, whereas the male has two different AMEL genes.[26] Amelogenin is also expressed transiently in differentiating odontoblasts during predentin formation, but is absent in mature functional odontoblasts. In intact adult teeth, amelogenin is not present in dental pulp, odontoblasts, and dentin. However, in injured and carious adult human teeth, amelogenin is strongly re-expressed in newly differentiated odontoblasts and is distributed in the dentinal tubuli under the lesion site.In an in vitro culture system, amelogenin is expressed preferentially in human dental pulp cells that start differentiating into odontoblast-like cells and form mineralization nodule.[27]

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.[28]

DNA FINGERPRINTING

DNA fingerprinting or DNA profile are encrypted sets of numbers that reflect a person's DNA makeup, which can also be used as the persons identifier. The DNA found can be genomic (found in the nucleus) and mtDNA (in the mitochondria). The non-coding DNA generally may either be as single copy acting as a spacer DNA between coping regions of genome or exist in multiple copies this is being called repetitive DNA (20 -30%). The repetitive sequence is highly polymorphic and unique to each individual. It appears as long tandem repeats (midi satellites), short tandem repeats (STR; mini satellites) and interspersed repetitive sequences.[4] Biological evidence generally means the comparison of genetic material such as DNA. But DNA analysis can be expensive and time consuming.[21]

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.[29] Sweet (2001) stated that the PCR method enables differentiation of an individual from another, with a high level of reliability and with about 1ng (one-billionth of a gram) of the target DNA.[30]

Murakami et al conducted a study in which sex determination using polymerase chain reaction (PCR) on tooth material was evaluated from the viewpoint of forensic medicine. The sensitivity of PCR for detection of the Y chromosome-specific alphoid repeat sequence and the X chromosome-specific alphoid repeat sequence was 0.5 pg of genomic DNA. Sex could be determined by PCR of DNA extracted from the pulp of 16 freshly extracted permanent teeth and dentine including the surface of the pulp cavity of 6 freshly extracted milk teeth.[31] PCR is an extremely sensitive technique but is prone to contamination from extraneous DNA, leading to false positive results. Another potential problem is due to cross-contamination between samples. Non-specific binding of primers and primer–primer dimmer formation are other possible reasons for unexpected results. Reagents and equipments are costly, hence can't be afforded by small laboratories.[32]

EFFECT OF ENVIRONMENT ON PULP

Shortly after the death of an individual, decomposition of tissue starts leading to a series of changes which can be observed morphologically and histologically. These changes occurring in a specific sequence can also be observed in dental pulp tissue retrieved from the jaws of buried dead bodies. There are a number of factors which have their interplay in this complete process of decomposition of tissue. Release of intracellular hydrolytic enzymes initiates the process of cellular disintegration. The rate of decomposition increases as the microorganisms invade these tissues.[33]

Duffy et al conducted a study to assess sex chromatin in artificially mummified and heated pulp tissue and found that sex chromatin (both Barr bodies and F-bodies) is shown to preserve in dehydrated human pulps up to one year and human pulp tissue retains sex diagnostic characteristics when heated to 100°C for up to 1 hr.[34] In another study by Duffy et al. 1991, stability of pulp nuclei was found to be ranging from 4 days to 2 weeks in the pulp tissue retrieved from coastal environment.[35]

Mehendiratta et al conducted a study to find out the series of various changes which occur during the process of putrefaction of the dental pulp in a coastal environment like that of Southern India and concluded dental pulp buried in a coastal environment goes through a specific series of morphological and histological changes which can be interpreted up to 144 h from burial, after which pulp ceases to exist.[33]

However, Suazo et al conducted a study to determine the effect of high temperatures on the diagnostic performance of the Barr chromatin observation on teeth and concluded that it was only possible to evaluate the samples from groups subjected to 200 and 400 degrees C.[36]

CONCLUSION

The need for personal identification arises in natural mass disasters like earth quakes, tsunamis, landslides, floods etc., and in man-made disasters such as terrorist attacks, bomb blasts, mass murders, and in cases when the body is highly decomposed or dismembered to deliberately conceal the identity of the individual. Because of the resistant nature of dental tissues to environmental assaults, such as incineration, mutilation dental tissues offer an excellent source in forensic analysis.The dental pulp is encased in a sturdy shell of hard tissues of the teeth and is easily retrievable. The estimation of sex is one of the pillars

of forensic identification. Therefore, utilisation of dental pulp for gender determination can play a cardinal role in forensic investigations.

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