Review Article

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Sex Determination Using Tooth Pulp: A New Tool in Human Identification

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Abstract

The use of biological evidence for identification of an individual is relatively recent development. Dental identification has long been considered a reliable method when other methods fail because of critical body conditions or unavailability of body parts. Tooth pulp is encased in a hard tissue where it may be protected from detrimental effects of impact, trauma, and heat. Casperson *et al* was the first to develop a technique using pulpal tissue stained with quinacrine mustard, specific for Y chromosome to determine sex of an individual. This article describes the use of dental pulp in sex determination both antemortem and postmortem.

Keywords: Tooth pulp; Pulp Tissue; Dental Identification; Forensic Odontology; Postmortem; Antemortem.

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]. Human identification is of paramount importance for both legal as well as humanitarian purpose [2].Dental identification has long been considered a reliable method when other methods fail because of critical body conditions or unavailability of body parts [3].The use of biological evidence for identification of an individual is a relatively recent development. Biological evidence generally means the comparison of genetic material such as DNA. However DNA analysis can be expensive and time consuming [4]. Teeth are the most durable organs in the body and can be heated to temperatures of 1608 ^{0}C without appreciable loss of microstructure. Teeth can survive long after soft and skeletal tissues have been destroyed [5].Tooth pulp is encased in a hard tissue casting where it may be protected from detrimental effects of impact, trauma, and heat [6]. Casperson *et al* developed a technique using pulpal tissue stained with quinacrine mustard, specific for Y chromosome to determine sex of an individual [7]. The histological method for sex determination involves observation of the presence of chromatin body or Barr body in somatic cells [8]. The Barr bodies were initially studied by Barr and Bertram in the nuclei of the nerve cells of cats. Since then the study of Barr bodies has been an integral part of exploration by researchers in the field of forensic odontology. The sex chromatin / Barr body observation is possible in various cells and used for identification of biological sex [9]. Duffy et al(1991) used experimentally dehydrated and cremated remains to isolate dental pulp cells for sex chromatin analysis [10].

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Method of Pulp Extraction

Examination of Patients and Extraction of Teeth

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Sectioning of Teeth

Modeling wax was folded and made into a block. The tooth was embedded on the modeling block. To free the pulp, the crown was separated longitudinally by using a carborundum disc at 30,000 rpm. Similarly,

the root was split for pulp removal. As described by Malaver and Yunis [11] the pulp was removed with a sterile curette and kept dry in a sterile test tube.

The pulp tissue was then transferred to the dry and clean conical centrifuge tubes containing 5 ml of fixative (3 Methanol:1 Glacial acetic acid) and left as such for about half an hour to 2 hours for the fixation of the pulp cells. It was then crushed with the glass rod sufficiently to isolate the pulp cells. A suspension thus obtained was centrifuged for 10 minutes at 1000 rpm. The supernatant was discarded leaving behind the pellet in the centrifuge tube. Five ml of fresh fixative was then added to re-suspend the pellet and the process was repeated thrice till a clear suspension of the pulp cells was obtained.

Staining Dental Pulp Cells

Thin smears were prepared on chilled microscope slides of 1 mm thickness by the air-drying method i.e. by dropping 2-3 drops of the above suspension on the slide from a distance of inches to get a homogenous population of cells. The cells were stained with 5% Quinacrine hydrochloride to stain the Y chromosome. The slide was mounted in buffer of PH 5.5 and was observed under Leica DMR fluorescent microscope (Oil immersion in dark field at ×40) by BV exciting method (emitting a blue-violet color, mainly at 4.047A0 and 4.038A0). Only those cells which contained the characteristic Y chromatin i.e. a brightly fluorescent spot attached to the nuclear membrane were counted as positive cells while those which did not show any such fluorescent spot were labeled as negative.

Or

The pulp tissue was transferred into small mortar with a needle and 0.5 ml of 20% acetic acid was used to soften the dental pulp which was then crushed to separate the cells. Again 2 ml of 20% acetic acid was added and the suspension was stirred well. Two drops of the suspension were placed in a cytofunnel clamped to a slide holder which was then placed into the cytospin (Shandon Inc, USA) and spun at 2,000 rpm for 5 minutes to obtain a monolayer of cells on a fluorescence microscope glass slide. After drying at room temperature, a few drops of absolute methanol were added to fix the material. After natural evaporation of the methanol, the material was stained with 0.5% quinacrine dihydrochloride for 20 minutes. The slide was then washed with double distilled water and kept in McIlvaine's buffer (0.1 M citric acid, 0.2 M dibasic sodium phosphate, ph5.5) for 3 minutes. The slide was then washed with 0.4 g/L magnesium chloride for 10 minutes and then a drop of glycerol was added and a cover slip was placed on top. The specimen was observed with a Leica DMR fluorescent microscope (Leica Microsystems, USA) under oil immersion in dark field at an objective of 40by BV exciting method (emitting a blue-violet colour mainly at 4.047 and 4.038 AÚ). Gender was identified as male if one fluorescent spot was observed in the nucleus (F-body, seen in the Y chromosome) and as female if no spot was observed.

Discussion

In a study done by Nirmal Das et al (2004) it has been shown that up to a period of four weeks after death we can determine the sex accurately from the study of X & Y chromosomes keeping in view the variation of temperature and humidity [9]. Wittakarand & Coworkers 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 [12]. In another study It has been shown that when chromosomes are stained with quinacrine mustard, they fluoresce differentially along their length when viewed under ultraviolet light and that the human Y chromosome fluoresces more brightly than the other chromosomes [12]. A similar study by Adachi [13] demonstrated the appearance of Y chromatin in the dental pulp stained by quinacrine mustard. He further added a decrease in the Y positive cells and decreased reliability of the study as the tooth ages was noted in the male teeth. In a study by Hiroaki Nogami et al (2008) [14] examined sex determination from dental pulp DNA using the loop-mediated isothermal amplification (LAMP) method. The study found that sex determination by the LAMP method was rapid and simple and it should prove useful in identifying victims of mass disasters. The X allele was detected in approximately 32 minutes with real-time turbid meter and the Y allele was detected in approximately 34 minutes. Analysis time was reduced to half when using loop primers. Visual detection was also possible as the amplified product showed white turbidity. The LAMP method amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions. It uses a DNA polymerase and a set of four specially designed primers that recognize six distinct sequences on the target DNA. This study used dental pulp samples from 32 unrelated Japanese subjects. Dental pulp samples were obtained from permanent

teeth that had been stored at room temperature for 1-25 years. A set of four specially designed primers was prepared based on the database from Gene Bank in order to detect X and Y alleles of the amelogenin locus. Also X-loop r and Y-loop f were also designed as loop primers. DNA amplification was performed using a loopamp DNA amplification reagent kit. Caspersson et al [7] suggested that alkylating agents such as quinacrine accumulate in DNA regions rich in guanine. This technique has been used in forensic science for sex determination from dried blood stains, saliva, and hair [12]. Thus this technique might be applicable to sex determination from tooth pulp some time after death because the teeth are a stable part of the skeleton and the pulp tissue is well protected. In 1972, Seno and Ishizu[15] carried out the detection of Y chromosome in the nuclei of dental pulp. They found that f- body positive rate in the nucleated cells of male dental pulp was over 30% even with the male teeth as old as 5 months after the extraction. With female teeth, typical f-body cannot be detected and f-body like spot has been observed in 0.4% of cells indicating that there can be no error in the identification of male tooth from that of female one, even such an f-body like spot is taken as an f-body itself. As the caries progresses, the inflammatory processes in the pulp are initiated as the inflammation progresses the internal architecture of the cell is lost and is also characterized by necrotic poorly stained cellular debris [16].

In a study by Sara et al [17], authors extracted DNA from the dentin and pulp of 14 teeth by using a silica-based methodology. They used the amelogenin gene to determine the sex via polymerase chain reaction. β-actin, a housekeeping gene was used as a control gene. The DNA yield depended on the type of tooth and was lowest in the smallest teeth i.e. incisors. In all cases, the authors were able to identify the sex as well as the control gene which suggests the potential to identify other genes such as short tandem repeats. It is possible to correctly identify a person's sex from dentin and pulp. Galdames et al(2010) conducted a study on histological sections of human dental pulp using Haematoxyline & Eosin stain and found out that the mean of Barr body - positive cells was 20.4 (SD 0.44) in female samples. There was no Barr body - positive cells in preparations of male subjects [8]. Das et al (2004) examined pulp tissue smears for determination of the sex of an individual. They reported that 24.92% of women pulp cells were positive for Barr body observation and that with an increase in post-mortem time number of Barr bodies decreased and sex identification was possible till 4 weeks. They also found that 36.5° of temperature is suitable for Barr body study and with further increase in temperature, the number of Barr bodies decreased [9]. In a study by Tsuchimochi T et al (2002), they 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 [18]. Hanaoka et al (1996) 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 [19]. Sivagami and co workers (2000) prepared DNA from teeth by ultrasonication and subsequent PCR amplification, and obtained 100 % success in determining the sex the individual [20].

Conclusion

Determination of sex hold a prime importance in mass casuality,wars, natural calamities, legal matters and immigration issues. Different method can be used for sex determination but with certain shortcomings, determination using pulp is a valid method because pulp is preserved even in adverse condition due to shell of hard dental tissue outside it.fluoresecnt dye method ,barr bodies and PCR methods can be used with pulp for determination however more advancement is required in this field.

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