Dental DNA Finger-printing in Identification of Human remains

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Abstract

DNA fingerprinting is a tool used to un-reveal all the mysteries associated with the oral cavity and its manifestations during diseased conditions. The technical advances in molecular biology have propelled the analysis of the DNA into routine usage in crime laboratories for rapid and early diagnosis. As dental pulp is surrounded by dentin and enamel, which forms dental armor, it offers the best source of DNA for reliable genetic type in forensic science. Unlike a conventional fingerprint that occurs only on the fingertips and can be altered by surgery, a DNA fingerprint is the same for every cell, tissue, and organ of a person. It cannot be altered by any known treatment. Consequently, DNA fingerprinting is rapidly becoming the primary method for identifying and distinguishing among individual human beings. Forensic dental record comparison has been used for human identification in cases where destruction of bodily tissues or prolonged exposure to the environment has made other means of identification impractical, that is, after fire exposure or mass disaster. The currently available DNA tests have high reliability and are accepted as legal proofs in courts. This review involves the use of DNA for human identification, and makes an overview of the evolution of this technology in the last years, highlighting the importance of molecular biology in forensic sciences.

Key Words: - Dental Specialties, DNA Profiling, Forensic Odontology.

Introduction

Forensic dentistry plays an important role in human identification in mass disasters, especially in fires, explosions, decomposing bodies or skeletonized bodies, when there is little material remaining to perform visual identification. It has led dentists working with forensic investigation teams to become more familiar with the new molecular biology technologies.[1]
Different techniques of identifying individual through dental means are available. Currently there are four types of personal identification circumstances that use teeth, jaw and Oro-facial characteristics, which include comparative dental identification, reconstructive post-mortem, dental profiling and DNA profiling.[2]

Dental identification:

This is the most common role of the forensic odontologist. Dental identification of a body may sometimes be necessary due to the circumstances of the death. It must be noted that the vast majority of deceased are identified by non-dental means, namely visual identification by a family member and fingerprint identification. But when neither of these are possible due to disfiguration or decomposition, that may render the deceased unrecognizable, or where people are not available to make a positive visual identification, then dental identification may be required. Confirming the identity of the deceased is not only important for the family and friends of the deceased from an emotional and grieving aspect but is also a legal requirement.[3]

Disadvantages of Comparative Identifications:

1. Discrepancy if person had additional dental treatment in time interval between the dates of ante-mortem and post-mortem dental records.
2. Poor quality of ante-mortem records.
3. Inability to locate and obtain suitable anti-mortem records.
4. Diseased person is not of same area.
5. Patient treated in emergency basis with no record.

Basis for DNA fingerprinting:

DNA:

Teeth present as an excellent source as DNA material[4] and its sources are pulp, dentine, cementum and periodontal ligament fibers. DNA from teeth and bone are preserved for many years even after putrefaction of remains[5] other sources include saliva and mucosal swabs. Saliva may also be isolated from various sources in the crime scene, for example, postage stamps and envelopes, glasses, cigarettes, straws, food and chewing gum, toothbrushes and dental floss, and dental impressions.[6,7] Use of DNA for human identification is proved to be very effective and has been documented.[8]

Basis for DNA Finger-printing:

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.[9] Gene is a segment of DNA that codes for a particular protein. This accounts for only 2-5% of entire cellular DNA. The function of the remaining 95% or more of the DNA is not known and is called as non-coding DNA or junk DNA. 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. The extreme variability in pattern of mini satellites detected by probe together with stable inheritance in usual Mendelian manner of individual pattern forms the basis of DNA fingerprinting. Variations in DNA sequence called polymorphisms can be used both to differentiate and to correlate individuals.[10]

Anatomical location for DNA in tooth:

The teeth differ in form and size but have similar histological structure. The dentin is a connective tissue that forms the major structural axis of the tooth and is hardly exposed to the oral environment. The dentin on the crown of the tooth is covered by enamel. The enamel has an ectodermic origin and is an extremely mineralized tissue. Furthermore, it is an acellular and avascular structure without nerves. The root dentin is covered by the cement, another type of calcified connective tissue. 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. Other less frequently used anatomical locations of DNA includes, odontoblastic process that extend into dentinal tubules, soft tissue within accessory canals, cellular cementum, adherent bone and periodontal ligament fibers.[4]

Polymerase chain reaction (PCR):

Polymerase chain reaction (PCR) technique allows amplification of DNA from even negligible amounts of source material.[11] The amplified DNA is then compared with ante- mortem samples such as stored blood, hairbrush, clothing, cervical smear, biopsy specimens. Other methods include Restriction fragment length polymorphism (RFLP), single nucleotide polymorphism-based (SNP) and micro-assays.[5] DNA can also help in identification of a parent or sibling.

Most of these techniques involve nuclear DNA but mitochondrial (mt) DNA is more abundant, and can be identified in cases when nuclear DNA is insufficient. Dental tissues like dentin and cementum are rich in mtDNA.[12]

Deoxyribonucleic acid and Forensic dentistry:

Due to the resistant nature of dental tissues to environmental assaults, such as, incineration, immersion, trauma, mutilation, decomposition, and microbial action, teeth represent an excellent source of DNA material.
In the tooth, dentin and pulp are rich sources of DNA, which can be successfully extracted.[13] The total production of genomic DNA obtained from the fresh dental sample may range from 6 to 50 μg DNA.[14] Sweet stated that the PCR method enables differentiation of one individual from another, with a high level of reliability, and with about 1 ng (one billionth of a gram) of the target DNA.[15] Thus, abundance of quality DNA can be extracted from a tooth, which is an important advantage in DNA analysis.[16] Deoxyribonucleic acid is preserved in the teeth and bones for a very long period and thus is a valuable source of information. Ancient DNA (aDNA) analysis can be carried out in samples that are hundreds to tens of thousands of year's old.[17]

Types of Deoxyribonucleic acid:

Genomic and mitochondrial are two types of DNA that are used in forensic sciences. The genomic DNA is found in the nucleus of each cell in the human body and represents a DNA source for most forensic applications. The teeth are an excellent source of genomic DNA.

Mitochondrial Deoxyribonucleic acid is another type of material that can be used when the extracted DNA samples are too small or degraded, such as those obtained from skeletonized tissues. The likelihood of obtaining a DNA profile from mtDNA is higher than that with any marker found in a genomic DNA.[18] Various biological samples such as hair, bones, and teeth that lack nucleated cellular material can be analyzed with mtDNA and it is very useful.

Applications of Deoxyribonucleic Acid Profiling in Forensic Dentistry:

The currently performed DNA profile tests are totally reliable and give details about an individual's physical characteristics, ethnicity, place of origin, and sex. These tests are also accepted as legal proofs in courts, for investigation of paternity and human identification.

1. **Restriction Fragment Length Polymorphism (RFLP)**

It is used for analyzing the variable lengths of the DNA fragments that result from digesting a DNA sample with a special kind of restriction enzyme called 'restriction endonuclease,' which sections DNA at a specific sequence pattern known as a restriction endonuclease recognition site. It may be difficult in samples degraded by environmental factors and also takes a longer time to get the results.[19]

2. **Short tandem repeat typing.**

It is described as a short stretch of DNA that is repeated at various locations throughout the human genome and this technology is used to evaluate the specific regions (loci) within the nuclear DNA (m). Each person has some STRs that are inherited from the father and some from the mother, however, no person has STRs that are identical to those of either parent. The uniqueness of an individual's STRs provides the scientific marker of identity, and hence, is helpful in forensic identification and paternity testing.[20] A short tandem repeat can be used for the identification of bodies in mass disasters and old skeletal remains.[21] Even though the DNA present in the ancient remains appear to be very degraded, it is conserved better in the tooth than in the bone samples.[22]

On the basis of STR, the Combined DNA Index System (CODIS) was established by the Federal Bureau of Investigation (FBI).[23] It was developed specifically for enabling the public forensic DNA laboratories to create searchable DNA databases of authorized DNA profiles. The odd chance that two individuals will have the same 13-loci DNA profile is about one in a billion. The United States maintains the largest DNA database in the world. The British data base for STR loci identification is the UK National DNA Database (NDNAD). The British system uses 10 loci, rather than the American 13 loci.

3. At times it is difficult to perform genetic identification with nuclear DNA due to the long-time interval between the time of death and examination of tissues. Usually in such cases only bone and teeth may be available for analysis. Teeth provide an excellent source for high molecular weight mtDNA, which offers several unique advantages for the identification of human remains[24]

Mitochondrial Deoxyribonucleic acid is a powerful tool for forensic identification as it possesses high copy number, maternal inheritance, and high degree of sequence variability. Each offspring has the same mtDNA as their mothers, as the mitochondrion of each new embryo comes from the mother's egg cell and the nuclear DNA is contributed by the father's sperm. In investigations involving missing persons, comparing the mtDNA profile of the unidentified remains with the profile of a potential maternal relative can be an important technique[23] However, mtDNA analysis is a slightly time-consuming technique and is exclusively matrilineal, and hence, less informative. Thus, this analysis is not usual in all forensic laboratories directed at resolution of crimes and identification of persons.

4. Y-Chromosome analysis:

Deoxyribonucleic acid polymorphisms on the human Y chromosome are valuable tools for understanding human evolution, migration, and for tracing relationships among males.[25] The majority of the length of the human Y chromosome is inherited as a single block in linkage from father to male offspring, as a haploid entity. Hence, Y chromosomal DNA variation has been mainly used for investigations on human evolution and for forensic purposes or paternity analysis.[26] Y-chromosome STR (Y-STR) polymorphisms are used in deficiency paternity testing, cases of physical assault, murders, sexual assault, and child abuse, where bite marks are frequently found on the skin.

5. X-Chromosome short tandem repeat:

The chromosome X-specific STR is used for the identification and genomic studies of various ethnic groups in the world.[27] As the size of the X-chromosome STR alleles is small, generally including 100 - 350 nucleotides, it is relatively easy to be amplified and is detected with high sensitivity. [28] X-chromosome STR (X-STR) markers form a powerful complementary system, especially in deficiency paternity testing.

6. Single nucleotide polymorphism:

Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome sequence is altered. For example an SNP might change the DNA sequence AAGGCTAA to ATGGCTAA.[29]

Single nucleotide polymorphisms have emerged as markers of interest to Forensic Medicine because of their small amplicon size, which is useful in analyzing degraded samples, lower mutation rate compared to STRs, amenable to high throughout analysis (automation), abundant in the human genome, can provide specific information about ancestry, lineage, evolution, identity or phenotype, and also determine sex.

Limitations of SNPs include, no widely established core loci and requirement of large multiplexing assays. Efforts are being made to investigate whether it can replace STR; nevertheless SNPs are the DNA technology of the future.

7. Gender typing:

The enamel proteins that are required for the development of normal tooth enamel are encoded by the amelogenin genes (they are part of a small group of genes that are active on both sex chromosomes).[30] The amelogenin gene is a single copy gene, homolog's of which are located on Xp22.1 - Xp22.3 and Yp 11.2. [31] The variation of length in the X-Y homologous amelogenin gene (AMELX and AMELY), are used for gender identification.[32] The gender may also be identified from the dental pulp DNA through the analysis of the peaks of X and Y loci by capillary gel electrophoresis.[33]

Conclusion:

Knowledge and importance of forensic dentistry is required for every dentist in injury and abuse cases for proper recording of findings to help investigating and legal officers. As forensic odontologist have a major role to play in identification of victims in mass disasters, a good quality of dental records makes the identification process easier. Teeth are unique and resistant to destruction and their records when well-maintained have a major impact in identification process. Dentists should also be aware of the legal aspects involved in forensic investigations and report to the concerned authorities to help in obtaining legal remedies to the victims. Meanwhile clinical observation of available medical and dental patient records remains the gold standard for forensic pathology.

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