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Dna Profiling – Saviour In Forensic Odontology

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ABSTRACT:

Forensic odontology is a branch of forensic science that is capable of human identification through dental tissues or more precisely teeth in cases where there is the destruction of body tissues in criminal investigations and mass disasters. Forensic odontology mainly involves dentists collaborating in legal and criminal issues. Parameters like age and gender identification are crucial in identifying the victim or victims. Through the past two decades, the molecular aspect of forensic sciences has expanded considerably, Molecular advancements in science like DNA analysis have expanded the reach of forensic dentistry, since teeth possess the ability to resist extreme environmental condition be it temperature, pressure or any chemical aggressions. Teeth are found to be the abundant supply for DNA, and hence it is an excellent source of genomic DNA. The present review focuses on molecular advancements in the field of forensic odontology as DNA analysis advances as molecular signature in forensic odontology.

KEYWORDS: *DNA, forensic odontology, mitochondrial DNA, polymerase chain reaction, short tandem repeat.*

INTRODUCTION:

For years the world has gone through several mass disasters such as bombings, terrorism, hurricanes, earthquakes, air crashes. World health organization (WHO) defines disaster as **"a sudden ecological phenomenon of sufficient magnitude to require external assistance"**. Traditional method of disaster victim identification include physical identification of documents, jewelry, and belongings. More trustable methods include review of dental data, hairs, and fingerprints. In 1985, Sir Alec Jeffrey published the first paper on identifying individuals based on DNA analysis through mini satellite hybridization [1]. The established importance of forensic dentistry for human identification gained a strong ground when there is little material to perform such identification as seen in most mass disasters. However, the use of DNA analysis in recent times gave a new direction to the scope of forensic dentistry. Owing to the unique property of gene that varies from person to person beside having genetic commonality, DNA profiling came out to be extremely useful. Except identical siblings, the DNA of every single individual is different. DNA identification tests use sophisticated techniques of molecular biology to compare samples taken from various sources such as: blood, semen, tissues, organs, bones, hairs, nails, teeth, saliva, urine and other body fluids. DNA can be isolated, as long as the sample contains nucleated cells, since every somatic cell contains the whole genome [2].

DISADVANTAGES OF VISUAL METHOD OF IDENTIFICATION:

Following visual method for identification is considered not reliable especially in large-scale disasters where the victims are mostly disfigured or beyond recognition [3]. The disadvantage lies in the fact that this method is mostly subjective, affected by cognitive anomalies and memory loss. Also, the visual method of victim identification is though authenticated because majorly it depends more on the emotional and the stressful situation through which a relative or victims' friend goes through [4]. So, it is always made sure and also as depicted by the Interpol guidelines- "victims should never be identified based solely on visual recognition method" [3].

IMPORTANCE OF DNA ANALYSIS IN IDENTIFICATION:

DNA analysis is a method which can also be done from fragments of victims. DNA analysis emerged out to be one of the main modes of victim identification in mass disasters[8].This DNA fingerprinting in identification happened to be an integral part of DVI management response in South East Asian tsunami incident [9]. Main advantage of this technique lies in the fact that DNA profiling or fingerprinting can be performed on a various body fluid and tissue types. DNA-based identification gained its popularity because of its greater percentage of success irrespective of the condition of the bodies in identification that is even if the body is decomposed, partially incinerated, DNA analysis can be carried out even irrespective of post-mortem interval and environmental conditions [7]. First DNA analysis was reported to be carried out in the mass disaster victims was during Scandinavian Star ferry in 1990. DNA-based analysis also played a very important role in victim identification in the Spitsbergen aircraft disaster in 1996, World Trade Centre attacks of 2001 and in South East Asian Tsunami disaster [6].

PRINCIPLE FOR DNA PROFILING:

The gene that codes for a particular protein contributes for only 2-5% of DNA while the remaining 95% are the junk or noncoding DNA. This non coding DNA may present as a single copy of spacer DNA or as multiple copies called repetitive DNA. The repetitive sequence exists as long or STRs. The variations in the mini satellite pattern that is detected by a probe along with stable inheritance forms the basis for DNA profiling. [8]

DNA-types

Genomic DNA - Teeth act as a good supply of genomic DNA. [24] They are present in nucleus of the cell.

Mitochondrial DNA - Used when DNA sample obtained is insufficient or degraded. [21]

Stages in DNA extraction

- Cell membrane rupture
- Denaturation of proteins using chelating agents and inactivation using proteinases
- DNA extraction.

DNA AND FORENSIC ODONTOLOGY:.

The unique property of DNA make it useful in forensic investigation. These molecules have the capability to withstand adverse conditions like temperature, pH, salt and other factors that usually destroy the classical serological markers. The three basic steps usually followed for DNA processing in forensic research are extraction, quantitation, and analysis and interpretation(Fig.1).

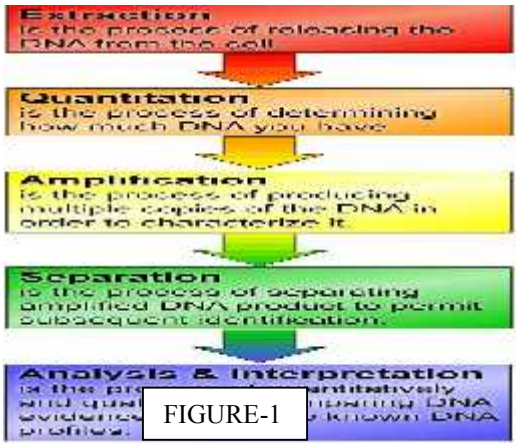


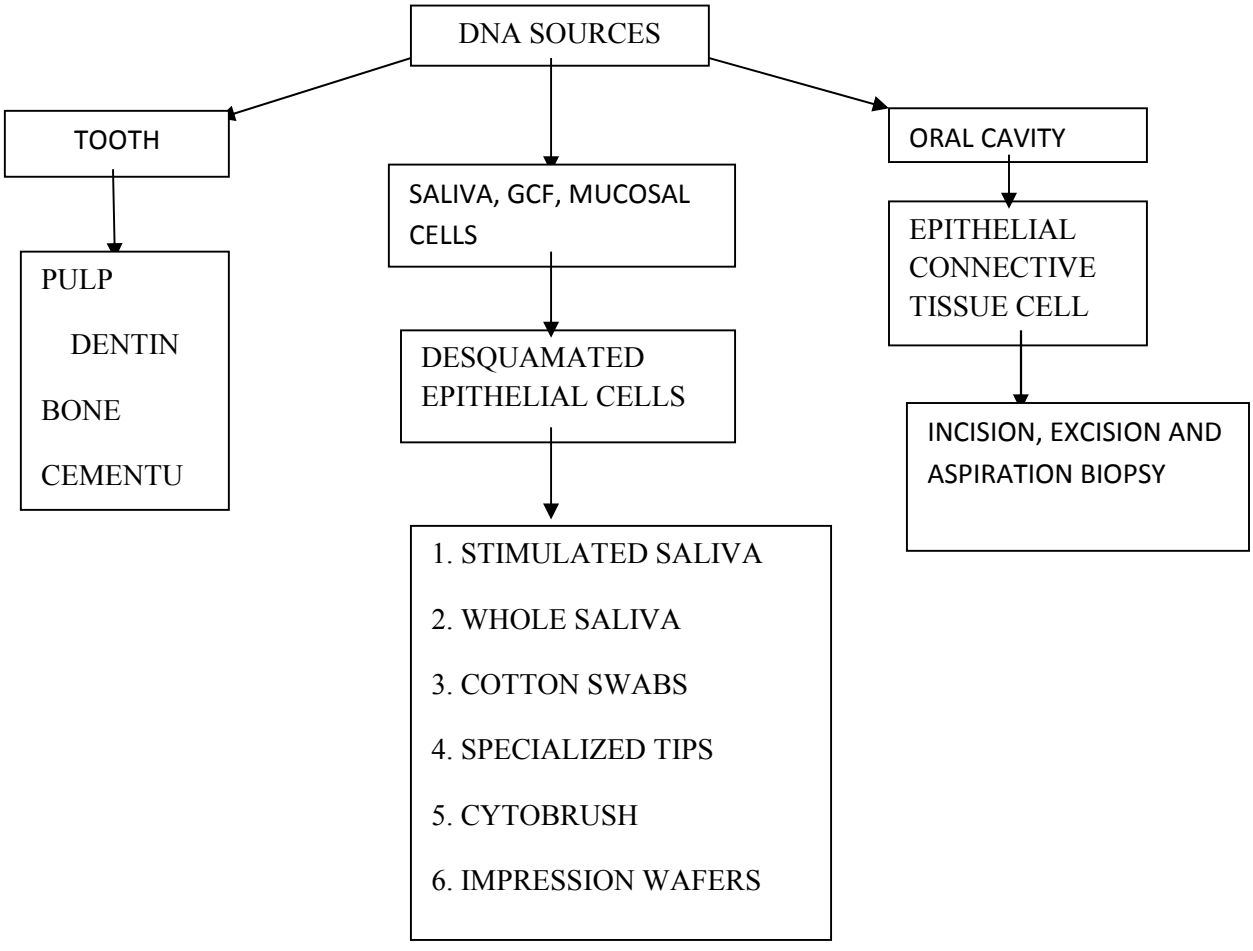
FIGURE-1

COLLECTION OF DNA

DNA can be obtained from various sources such as (cells that contain nucleus) blood, semen, tissues, bones, hair, nails, teeth, saliva, body fluids etc. The pulpal tissues in the teeth are rich in genetic information due to the availability of a higher number of nucleated cells. Isolation of Nucleated cells can be done from the adjacent bone, periodontal fibers and blood.

TEETH AS UNIQUE AND IDEAL SOURCE OF DNA: -

Teeth are uniquely resistant to physical and/or chemical aggression such as incineration, trauma and decomposition [4]. The chance of contamination or degradation of DNA is large with any tissue sample except the tooth.



Stravinos et al proposed guidelines to obtain dental DNA [5] [Table 1].

TABLE-1	Guidelines to obtain dental DNA
1	Determine if there is any soft tissue or blood adherent to the tooth that should be sampled.
2	Debride the tooth of any plaque or calculus with hydrogen peroxide.
3	If tooth is intact, a conventional endodontic access can be conducted.
4	Sectioning the tooth provides a greater access to the pulp chamber (vertical).
5	Once the tooth is opened, the walls of pulp chamber can be curetted. Pulp tissue and powder can be collected over a wide mouth sterile container

6	Tooth crushing may be performed.

DNA is generally degraded into fragments through autolytic and bacterial enzymatic action but information is not completely lost since they are present within the DNA fragments. Excessive DNA fragmentation during the decomposition process may result in the loss of genetic information and thus, becomes less useful in the forensic investigation [6]

METHODS TO PROCURE DNA FROM TEETH: TABLE-2

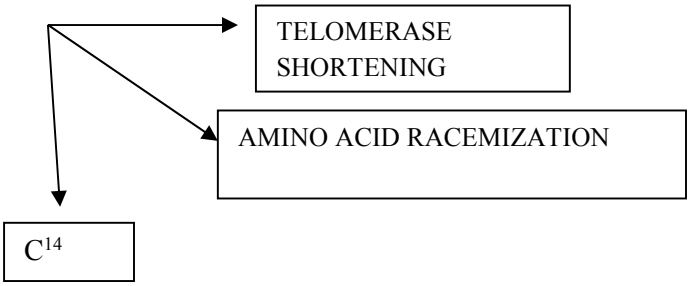
METHODS	
MILLING METHOD	• Destruction of morphological structure of ancient teeth • Used in anthropological evaluation
CONVENTIONAL ENDODONTIC METHOD	• Access of pulp chamber and dental pulp is retrieved.
HORIZONTAL SECTIONING	• Horizontal section of tooth with aggressive pulpectomy and crushing of radicular half of tooth. • Horizontal section of tooth with partial extirpation of coronal and radicular half of tooth.
CRYOGENIC GRINDING	
HORIZONTAL SECTIONING	• horizontal section of tissue with partial extirpation of coronal and radicular half of tooth

MOLECULAR TECHNIQUES IN DNA ANALYSIS

TECHNIQUES	
1.RESTRICTED FRAGMENT LENGTH POLYMORPHISM	• DNA strands are cut in fragments with a restriction enzyme. • fragmented DNA strands are electrophoresed on an agarose gel • Transferred to a nylon membrane. • Incubation with a radioactive probe consisting of complementary pieces of polynucleotides that can move and hybridize with DNA fragments
2. POLYMERASE CHAIN REACTION	• Highly sensitive molecular technique. • Quicker and less labour intensive. • Potential cross-contamination is the only disadvantage of this procedure[12] • Five main steps in PCR are initiation, denaturation, annealing, elongation and extension.
3. SHORT TANDEM REPEATS	• Core repeat sequences greater than seven

	base pairs have been called long tandem repeat or mini satellite. • Shorter STR fragments are generally preferable for a variety of technical reasons [13]. • that dentine provided a genetic profile of mitochondrial DNA and STR in the study sample[14]
4. MITOCHONDRIAL DNA	• Human mitochondrial DNA (Mt DNA) exists as a double-stranded circle containing 16.6 kb DNA. • It can be suggested that Mt DNA can be considered in investigation, if the nuclear DNA is not available [15] . • Its mode of inheritance, is the unique feature as Mt DNA is inherited in a strictly mother to-child manner, there is no paternal contribution. • So, no recombination and since only a single copy is present in the cell, an exact sequence match is anticipated. • Mitochondrial DNA sequencing has great application to severely decomposed remains [15, 16].

DNA PROFILING AIDING IN AGE ESTIMATION



TELOMERE SHORTENING:

The terminal end of human chromosomes is termed as the telomere. It possesses repeating sequences of TTAGGG. With each cell division,telomere is shortened. Telomere shortening results during the ageing process of many cells. Thus using DNA technology, age estimation can be done from dental pulp DNA based on shortening of telomere length [17].

AMINO ACID RACEMIZATION:

The biochemical changes associated with the chemical reaction where Levo - (L) amino acid is transformed into Dextro - (D) amino acids or vice versa which often detect slow metabolic activity. This method is considered to be accurate and estimates within ± 3 years of actual age. Racemization of L- to D-forms correlates highly with age. With advancementof age, the L-form converts to D-form [predominantly aspartic acid] [18].

^{14}C LEVELS

This method finds the amount of carbon 14 isotope present in enamel and compares it to recent atmospheric levels. The estimated age from this technique is around ± 1.6 years of actual age [19].

IN GENDER DETERMINATION:

AMEL gene :This gene codes for a highly conservative protein called "amelogenin" which is located on the X and Y chromosomes in human allosomes. These two alleles found to be similar in exonic sequence, but differ in intronic sequence. Female chromosome (XX) consists of two identical genes (AMEL), whereas male chromosomes (XY) have two unidentical genes [AMEL] [19]

IMPORTANT POINTS TO BE KEPT IN MIND WHILE GOING THROUGH DNA PROFILING:

- Severely charred bodies should be considered unsuitable for DNA analysis [20]
- Victim identification by DNA includes collecting appropriate antemortem and post-mortem samples, matching and statistical weighting of the genetic match [6]
- Antemortem samples include buccal swabs, Guthrie cards, and pathology specimens [20]
- Antemortem samples should be obtained from more than one first-degree relative. Also it can be obtained from the personal objects of the deceased.

SAMPLE COLLECTION

- Proper labeling, documentation, and proper chain of custody are crucial for collecting the samples.
- Samples for DNA analysis should be kept cool or certain DNA preservatives like genofix, sample matrix, regular salt, 95% (alcohol or white rum can be used).
- Post-mortem DNA samples in the case of teeth- molar teeth is preferable.

PRECAUTIONS WHILE COLLECTING AND HANDLING SAMPLES FOR DNA ANALYSIS: -

- Utmost care should be taken that samples are not contaminated. Contamination can occur from someone handling the sample, contaminated instruments or from commingled remains.
- The sampling area should be clean and protective clothing should be used while handling the samples.
- Samples should be stored under appropriate conditions in appropriate containers.
- Clean disposable instruments should be used.[6]
- All samples should be labeled, individually wrapped in packing material with a chain of custody details.

SOME OF THE INCIDENTS REPORTED WORLDWIDE WHERE DNA PROFILING PLAYED A VITAL ROLE:

In the Kaprun cable car fire disaster on November 11, 2000; 155 people were reported dead. It was impossible to do morphological identification because of the severe burning of bodies. But DNA analysis served as the saviour as all the remains were identified successfully through DNA analysis within 19 days after the incident. In the Yakolev-42 aircraft accident in Trabzon, Turkey of May 26, 2003 where DNA profiling was made one year later to confirm the identification of the remains. Again in the Madrid terrorist attack which happened on March 11, 2004, 191 individuals were reported dead. 220 remains were analyzed by DNA analysis. Also, mitochondrial DNA analysis was done for investigation in one case. DNA analysis successfully resulted in 100% identification of victims. In Tsunami disaster that occurred on 26 December 2004, in Thailand, Indonesia, and Sri Lanka, most of the victims were reported to be identified using dental and fingerprinting data and some were identified by DNA analysis. Skinner et al. 2010, mentioned Yugoslavia conflict in their article that happened between 1991-1999 , Out of 30000 missing individuals, two-third of the 15000 victims were identified through DNA. Interestingly 23% of 3919 remains were identified through dental information [22] Manhart et al. 2012, in their

study mentioned about the "Autobahn A19" disaster. Among the 8 victims which were found, 7 victims were identified by DNA analysis. Akhteruzzaman et al. 2015, in their article mentioned disaster victim identification by means of DNA analysis in Tazreen fashions garment fire incident. Out of 59 unidentified dead bodies, 43 were confirmed by DNA analysis with the help of 68 biological relatives originating from 61 families were reported. [23]

MERITS, DEMERITS, JUDICIAL VALUES OF DNA TECHNOLOGY AND TEST RESULTS:

The major advantage of the PCR technique is that it is quicker, and can be performed on degraded samples which can yield highly sensitive results. The disadvantage of this technique is the potential for cross-contamination. Mitochondrial DNA procedures can be performed on samples where the availability of the nuclear DNA is minimial. Although amelogenin helps in gender identification, the major disadvantage is the deterioration of the nuclear material with the passage of time [28] DNA test results having wide range acceptance and are having broad support. If two profiles are different, a person can be excluded with certainty. If two identical DNA sequences are found, a statistical approach has to be made to determine the frequency of a particular sequence at a specific locus in that population. However, the most frequent issues that is raised during the legal conversations are 1) quality assurance of testing procedures, 2) adequate genetic interpretations, and the interference of unfairness to defendants [25, 26].

DNA AND MASS DISASTERS:

In 2005 Alonso et al, in their paper had highlighted the challenges associated with DNA analysis in mass disaster investigations.A combination of proper management, analysis, and comparison among large numbers of biological samples and DNA profiles are essential in Mass Disasters. Along with that, it requires software with bioinformatics and statistical tools for searching DNA database and likelihood ratio calculations. Analysis of 13-17 nuclear STR markers should be done from family reference samples as follows- (a) either single or from both biological parents of the victim, (b) biological mate of the missing or unidentified person and their child/children, and (c) multiple biological full siblings (sharing the same parent as the victim).Recommended samples be Buccal swabs and blood for both nuclear and mitochondrial DNA analysis.[27]

RECENT ADVANCES THAT AID DNA ANALYSIS:

Technologies that aid in genetic identification such as mRNA analysis, microarray techniques, oligonucleotide microarrays, next generation genome sequencing, microfluidic systems, nanotechnology; Fluorescence in situ hybridization (FISH) probes specific for X and Y chromosomes which can differentiate between male and female cells; transcriptome variations in individuals and populations. [1,24]

CONCLUSION: The use of DNA technology in forensic sciences is established and the particular interest of providing DNA from the hard tissue remains of the body is extended to the tooth in forensic odontology. The tooth is considered to be the storehouse of DNA, thus, exploring the procedures of acquiring DNA from teeth can extend the reach of molecular advances in forensic sciences and strengthen the view of DNA as "a molecular signature in forensic odontology".

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