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Review Article

DNA – A DIAGNOSTIC TOOL IN FORENSIC SCIENCE

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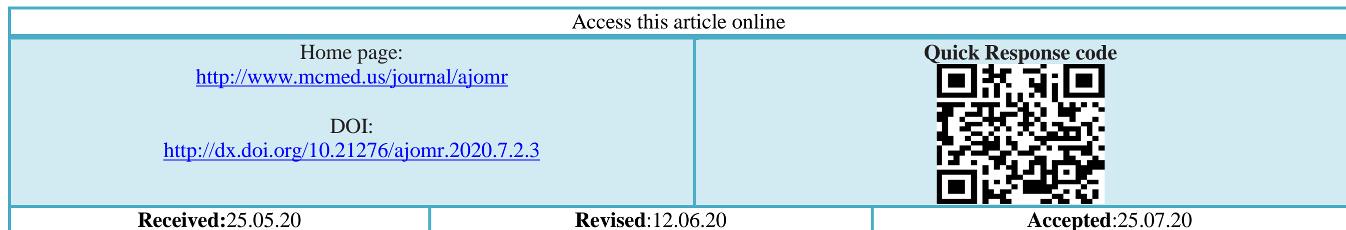
ABSTRACT
The recent trends in molecular biology have transformed all prospects in dental field. DNA, the language of life supplies information both in well- being or illness. DNA identification and analysis is a used to disclose all the enigmas related with the oral cavity during debilitated conditions. It is being extensively used in investigated many events associated with forensic dentistry. Recent advances in molecular biology have forced the DNA analysis into frequent use in crime departments for fast and immediate diagnosis. DNA is an outstanding means for recognition of unrecognized human dead remains. As pulp is enclosed by dentin and enamel, which acts as dental shield, it provides the good source of DNA for trusted genetic type in forensic odontology.

Key words:- DNA identification, Molecular biology, Forensic odontology.



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INTRODUCTION

Forensic dentistry labeling is at advanced track in medical field. The role of restorations, prosthetic device and radiographical labeling as the vital stray of forensic dentistry has descended lately, whereas molecular biology are swiftly expanding in efficiency and availability [1]. The tooth is the best relevant source to extract DNA because it is a packed box protecting DNA from extensive environmental situations, except its apical entry. This has persuaded the investigation of many human hard and soft tissues as probable source of genetic evidentiary key factor. Nowadays, teeth have been the substrate for DNA studies as the dental hard tissue encompasses the pulp and provides an anatomical contour of great durability[2]. Besides, when morphologically assessed, even a single dental unit provides relevant information concerning the individual to whom the tooth belongs [3-5].

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LITERATURE REVIEW

Jeffery (1985) described hyper variable regions of human DNA using multi locus probes and the applicability of these DNA polymorphisms to the individualization of human blood and tissues. The potential forensic applications of DNA analysis in resolving disputed parentage problems, identification of human remains as well as in the individualization of blood and body fluids in crime laboratories were immediately recognized.

Polymerase chain reaction (PCR), originally introduced by **Saiki et al.** and subsequently automated by **Mullis and Faloona,** has emerged as a powerful tool in forensics for the exponential in vitro amplification of specific sequences DNA or RNA and was rapidly applied to forensic odontology.

Schwartz et al. in 1991 isolated high molecular weight (HMW) from the teeth under different environmental conditions such as varying pH, humidity, temperature, storage, etc. It was determined that the environmental conditions examined did not affect the ability to obtain HMW human DNA from dental pulp.

Pötsch et al. in 1992 performed genomic dot blot hybridization for sex determination using the biotinylated repetitive DNA probe pHY 2.1 and sex was correctly classified in all cases using 50-100 ng target DNA from pulp.

Role and Responsibilities of Odontologist

- Collaborating with the laboratory and their capabilities, techniques and experiences.
- Becoming familiar with the contact individuals and DNA procedures.
- Consulting on all matters of dental and salivary evidence.
- Examining the evidence (tooth or salivary sample) thoroughly and documenting the circumstances under which the sample was obtained.
- The documentation should include photographs and/or radiographs where appropriate. These may be supplemented by sketches and a thorough written description of the location and identifying features of the evidence , temperature , humidity and potential sources of contamination should be noted as well.

Sources of DNA in Oral Cavity

- The tooth is the most important source to extract DNA. It is a preserved package of DNA protecting it from adverse environmental conditions [6].
- DNA extracted from the teeth of an unidentified individual will be compared with DNA isolated from known antemortem samples, such as stored blood, toothbrush, hairbrush, clothing, cervical smear, biopsy, or DNA of a parent or sibling.
- In the tooth, dentin and pulp are rich sources of DNA. Sectioning the tooth provides greater access to pulp. Once the tooth is opened, walls of the pulp chamber can be curetted or instrumented with a slow rotary burr. Then pulp tissue can be collected in a wide open sterile tube. In case of dried specimens, the pulp may be mummified parchment-like. After instrumentation, the chamber is best irrigated with buffer. Ultrafiltration of the liquid at the lab will remove the cellular material needed for analysis**.** [6]
- DNA can be extracted from the crown body, root tip, and root body. The root body yields the highest quantities of DNA.

Guidelines for Procuring Dental DNA

- Determine if there is any soft tissue or blood adherent to the tooth that should be sampled.
- Debride the tooth of any plaque or calculus with a curette and wash thoroughly with hydrogen peroxide followed by ethanol.
- If the tooth is intact (unrestored, non – curious, unbroken)and is believed to have been removed from the alveolus recently, a conventional endodontic access and instrumentation can be conducted.
- Sectioning the tooth provides a greater access to the pulp chamber (vertical axis sectioning).
- Once the tooth is opened, the walls of the pulp chamber can be curetted or instrumented with a slow speed rotary bur. Pulp tissue and powder can be collected over a wide – mouthed sterile container. In dried specimen, the pulp may be mummified, parchment – like or consist of wispy strands of tissue contracted against the chamber wall. After instrumentation, the chamber is best irrigated with TE buffer. Subsequent ultrafiltration of the liquid at the laboratory will remove the cellular material needed for analysis.
- Finally, crushing the tooth may be necessary.
- The forensic odontologist have to reveal all the procedures he executed and all the findings he acquired from the research to the contributor.

Variables Affecting Amount of DNA in A Tooth

- Type of tooth (incisor, canine, premolar or molar)
- Condition of teeth prior to extraction (degree of decay)
- Condition of tooth following trauma
- Period of time from extraction to DNA isolation
- Age of the individual
- Temperature
  - Increase in temperature → decreases the amount of DNA from the tooth pulp.
  - Decrease in temperature → does not cause any change in the quantity of DNA. [7]

Sampling Methods

Sampling is directed at collecting the sample tissue from which DNA can be probably extracted. Various techniques had been utilized to acquire the tissue causing limited damage to it. The amount of DNA exclusively depends on the sampling methods. Dentine is a very rich source of mitochondrial DNA and can be segregated from crown and root, so appropriately the source should be used. It is suggested to subsample the tooth, focusing the tissue of interest without much loss of

tooth structure and also an importance on the preservation of the tissue for future researches.

Destructive

- Crushing entire tooth.
- Conventional endodontic access.
- Vertical split of entire tooth.
- Horizontal sectioning.
- Cryogenic grinding.

Non Destructive

- Incubating the Sample in EDTA and Proteinase K.
- Soaking of Sample in Guanidinium– Thiocyanate (GuSCN) [5-8].

DNA Extraction Methods

There are different methods and techniques which are available to recover genomic DNA. Various traditional methods gave successful results in regard with isolation of DNA but they usually lack standardization and therefore it affects the yield. In general all the DNA isolation methods i.e. separation of the DNA from the cellular components involves disruption and lysis of the starting materials followed by removal of proteins and contaminants and finally recovery of the DNA [9].

Preparation of Crude Lysates

In this particular technique, the cell lysates are incubated at 900 C for 20 minutes, or proteinase K digestion is performed over cell lysates. The treated lysates are often not at optimal pH and contain contaminants which can lead to negative results and high failure rates.

Salting out Methods

It is a conventional technique where salts like sodium chloride, EDTA potassium acetate, ammonium acetate are used to precipitate the proteins and contaminants from the cell lysates. The precipitates are removed by centrifugation and the DNA is isolated by alcohol precipitation. The removal of contaminants using this particular method is not very efficient and often required repeated alcohol precipitation for gaining a proper yield [9].

Organic Extraction Methods

This technique uses organic solvents like phenol, chloroform and isoamyl alcohol to extract out the contaminants from the cell lysates. The correct salt concentration and pH must be used during the procedure to ensure that the contaminants are separated and DNA remains in the aqueous phase and after that by alcohol precipitation the DNA is extracted. It is highly impossible to automate this procedure and it generally generates toxic components as waste and hence hazardous to environment.

Cesium Chloride Density Gradients

This is a method of genomic DNA purification through the cesium chloride density gradient. The lysates are alcohol precipitated; the re - suspended DNA is mixed with cesium chloride and ethidium bromide and centrifuged. The DNA band is collected from the centrifuge tube, extracted and precipitated with ethanol to recover the crude DNA. This technique is expensive, time consuming and technique sensitive but yield a very high quality of DNA.

Anion-Exchange Methods

The principle behind this technique is based on the interaction between the negatively charged phosphates of the nucleic acid and positively charged surface molecules on the substrate. Solid phase anion exchange chromatography is used in this procedure. DNA gets bound to the substrate under low salt conditions and the impurities and other metabolites are washed away using medium salt buffers and simultaneously the DNA is eluted using high salt buffers. This DNA is finally subjected to all applications. The main advantage lies in the fact that it completely avoids the use of toxic substances [9].

Silica based Extraction Methods

This technique uses the silica gel membrane to selectively adsorb the nucleic acids in the presence of salts. Optimized buffers ensures only DNA is adsorbed and other impurities are washed away. This is comparatively more effective and efficient than others. DNA is obtained from the silica gel membrane using low salt buffer. There is no precipitation of alcohol is required and DNA re -suspension is not required like other extraction methods. Various readymade kits are commercially available for rapid isolation of DNA from a variety of sample sources. It is suited in various applications like polymerase chain reaction techniques, restriction fragment length polymorphism.

Chelating Resin Based Extraction

It is also known as chelex - based DNA extraction. DNA that is extracted using this technique can be amplified by using PCR. In this method there is a lesser chance of clinician introduced contamination of exogenous DNA into the sample mix and hence the yield of quality DNA is more.

Applications of DNA in Forensic Odontology

Short Tandem Repeat Typing

Short tandem repeats (STRs) are identified as mini chains of DNA that are repeatedly seen at various region in the DNA chain and the specific loci within which the DNA are identified. STRs can be used to predict the disease links in the family, retrieve the

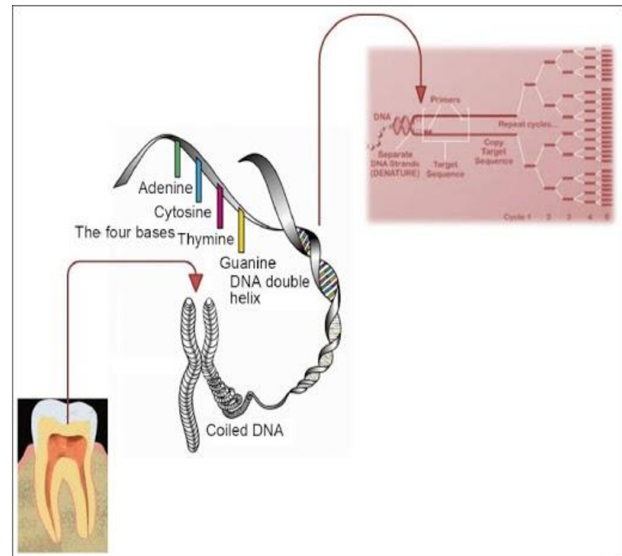
familial identity and also be helpful in anthropological studies. These STRs are derived from parents and hence they are unique in their characteristics. A study in Serbian population shows that specific autosomal loci (STRs) from teeth was found to be useful in revealing identities of the individual from a particular population. Even though the DNA is degraded in the bones of the ancient remnants, it is well conserved within the tooth. Combined DNA index system (CODIS) was established by Federal Bureau of Investigation (FBIUSA) on the basis of STRs and helped in creating a large DNA based database [9].

PCR methods

PCR is used to amplify the amount of DNA material available, so that sufficient quantity is available to carry our DNA analysis. To carry out the reaction special enzyme and DNA primers are required. These primers are like probes with known constant sections of DNA but not labeled. They are designed to known constant sections of DNA at the ends of variable region to be amplified. The principle of PCR is that the DNA is capable of duplicating itself. This is done by unwinding the strands of DNA and each strand acts as a template for synthesis of new strand. By PCR technique we can amplify specific DNA segments dependent on the primer employed. The standard PCR reaction runs through 30 cycles in a couple of hours which results in amplification of original DNA by over 109 times.

The DNA found can be genomic (found in the nucleus) and mtDNA (in the mitochondria). The teeth are an excellent source of genomic and mtDNA because PCR analyses allow comparing the collected postmortem samples to known antemortem samples or parental DNA. Main advantage of mtDNA is the high number of copies per cell (from hundreds to thousands of organelles) [10].

Figure 1: Schematic photograph showing replication of DNA by PCR



mtDNA analysis

mtDNA differs from nuclear DNA in its location, its quantity in the cell, its mode of inheritance and its sequence. mtDNA analysis can be used to examine the DNA from samples that cannot be analyzed by RFLP or STR. mtDNA analysis uses DNA extracted from another cellular organelle called a mitochondrion. While older biological samples that lack nucleated cellular material, such as hair, bones and teeth, cannot be analyzed with STR and RFLP, they can be analyzed with mtDNA. In the investigation of cases that have gone unsolved for many years, mtDNA is extremely valuable. It is better than nuclear genome as it is passed through maternal lineage and has 100-1000 copies of mtDNA genome. This analysis can be used in the tooth especially dentin and cement which contain enough DNA to allow the amplification of the mtDNA, which can be used in the human identification [10].

Restriction Fragment Length Polymorphism (RFLP)

It is an application by which the differences in the DNA sequences which are homologous in nature and can be detected by the identification of fragments at different length. The DNA is broken down into fragments by the cleavage enzyme known as restriction endonuclease. Most RFLP are highly locus specific. It signifies a labeled DNA sequence that gets hybridized with more than DNA fragments. Finally it is subjected to the gel electrophoresis and a unique blotting is revealed which denotes a definite genotype at a specific loci. There are different types of RFLP probes like short, single or low copy genomic DNA or complementary DNA. The application of these varies from gene mapping, genomic studies, phylogenetic tracing, diagnosis etc. Extraction of sufficient DNA for RFLP analysis is time bound and technique sensitive. However, PCR helps in amplifying very small amounts of DNA to the quantity required for RFLP analysis. Therefore, more

samples can be examined in a shorter span. An alternative terminology for the technique is Cleaved Amplified Polymorphic Sequence (CAPS) assay. DNA from ancient bones and teeth were extracted analyzed for RFLP, it provided a good alternative to sequence the PCR products, further it helped in differentiating species even in cases of destroyed DNA template or minimal quantities of DNA availability [9].

X and Y Chromosome Short Tandem Repeats

DNA polymorphisms are valuable tools for tracing out human evolution, migration and familial relationships. The Y chromosome is inherited from father to male offspring as a haploid .The size of the X chromosome short tandem repeats alleles are small and hence they are relatively easy to amplify and study.

Single Nucleotide Polymorphism (SNPs)

They have emerged as markers of interest because of its small size .The most primary fact is that SNP need a smaller target and a single nucleotide need to be investigated rather than numerous nucleotides. It is useful in analysis of DNA from degraded tissue samples and are better than STRs. In a particular study genetic disturbances associated with MSX1 and PAX9 genes were studied using SNP and hence play a definite role in tracing a race or a group of endangered population and also phylogenetic studies. The challenges which exist in SNPs is that it needs multiple detection levels and the standardization of the markers and still the application of STR versus SNP is debatable.

Human identification using the morphometric features of the teeth have been used since ages. The use of tooth DNA has added an advantage to the existing identification modalities. Minimal quantities of DNA retrieved from site of investigation also hold promise to render accurate results. The extraction of mitochondrial DNA has always been more advantageous as it is more stable with lesser variations when compared to DNA derived from nucleus. Hence will yield better results in solving forensic investigations [9,10].

CONCLUSION

Tooth is an abundant source of DNA, and can be utilised for many forensic reasearches. The remarkably rich source of DNA from tooth is dental pulp as it is rich in cells and also very easily retrievable from the tooth structure for DNA isolation. Efforts have been made to retrieve DNA from cementum and dentin in case dental pulp is affected. In order to assist forensic odontologist in tracking the individual and also to provide justice to the affected victim, a protocol of DNA sampling from various tooth tissues and also the procedure of analysis should be followed.

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