Review Article

Forensic genetics: Scope and application from forensic odontology perspective

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Abstract

Forensic science corresponds to the employment of science to the law or legal matters. One of the major concerns of legal system is to deduce the recognition of an item or individual, involved in crime for which forensic expert plays a pivotal role. Forensic odontology, a budding branch in dentistry, involves the application of dentistry to the legal system. The dental characteristics are considered as one of the primary characteristics of identification as per Interpol DVI guidelines. Thus, establishing the identity of unknown human remains through dental features is considered as one of the core domains of forensic odontology. However, its reliability and its acceptability in the court of law are only secondary to the application of DNA technologies. Also, the acceptability of bite-mark analysis and its evidentiary role is debatable. However, the bite marks may also be a source of salivary DNA, to establish the linking of the perpetrator to the victim. The recent advancements in the DNA technologies and the use of teeth and saliva as sources of DNA are the added advantages in the application of DNA as person identifiers especially in badly mutilated, decomposed and charred bodies and in linking the perpetrator to the crime. With this background, we present here a review on the application of forensic genetics from a forensic odontology point of view.

Keywords: DNA isolation, DNA profiling, forensic genetics, forensic odontology, saliva, teeth

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BACKGROUND

Forensic odontology incorporates numerous spaces of study, where the legal framework and dentistry overlap. It is a perceived expansion of dentistry which, in the legal executive, manages appropriate taking care of and examination of dental confirmations, and with the real assessment and documentation of dental confirmations.[1] To name a few, the forensic odontologists play an important role in investigations under CrPC 174 for establishing the identity of the deceased, under IPC 375 involving sexual assault for bite-mark analysis, under IPC section 320 for causing grievous injury, under POCSO Act 2012 and Child Labour Act, 1986 for dental age estimation. In cases, under the Indian Evidence Act, 1872, the forensic odontologists also assist the judicial system by relating expert testimony concerning the dental evidences and giving an opinion in the court of law. The uniqueness of dentition, the class and individual qualities of teeth in addition to the openness of ante-mortem dental archives are the explanations behind the fruitful contribution of legal odontology in

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the acknowledgment of human leftover in singular cases or in mass catastrophes. Also, the teeth are resistant to post-mortem changes and other environmental assaults like extremes of temperature, trauma and immersion in water bodies, and hence, they regarded as a very good source of DNA material.[2] According to the Interpol's DVI guidelines, the dental features are considered as one of the three primary characteristics of identification.[3] However in India, the application of dental parameters in person identification is not well established in real-life scenarios. This is probably because of the lack of awareness among the stakeholders in the police and the legal system, lack of a recognized disaster victim identification team with a qualified forensic odontologist as one of its member and lack or poor maintenance of the ante-mortem dental records in the clinical dentistry domain in the country. Hence, the police and the legal authorities depend solely on the secondary characteristics like the personal belongings, tattoos, etc., and/or in combination with the DNA analysis for confirming the identity of an unknown deceased. There are instances where the teeth are the only source of DNA and even at the time the forensic odontologists or the general dentists are not involved for the extraction of the 'ideal tooth' for DNA. The DNA analysis has proved to be vital in linking the perpetrator to a crime scene, say, for example, in the sexual assault case involving bite marks, the DNA evidence may be collected from the assailant's saliva trace evidence in the indentation (bite mark) on the victim's body. It is apt to mention at this stage that the immediate collection of saliva sample from the bite mark of assault (if left on victim's body) for DNA analysis is equally important to collecting the vaginal swab for DNA evidence. Hence from a forensic odontology perspective, the tooth and saliva are significant wellspring for forensic DNA investigations. The objective of this review paper is to highlight the basics of forensic genetics and to mention those arenas where the DNA technologies are applicable under forensic context like person identification in cases of mass disaster, age assessment and identification of suspect.

Basic Genetics and its application in person identification

DNA is the molecule where the biological information related to life is encoded. Prior to the conceptualization of DNA, an Austrian monk, Gregor Mendel (1822–1884) emphasized on the passing of traits within the generations. Genetics is the study of heredity and variations in organisms resulting owing to genetic makeup. The genetic characters carry forward to next generation determine the appearance, influence genetic diseases and may also affect behaviour.[4] In humans, the genetic information is stored in 22 sets of chromosomes (autosomes) and the leftover pair of two sex chromosomes, XX (female) or XY (male). The DNA of most extreme significance to a legal researchers are to be specific nuclear DNA (nDNA), mitochondrial DNA (mtDNA) and Y chromosome DNA.[5] The pattern of inheritance of DNA is such that both the parents contribute half of the autosomes, i.e., 23 from each parent. Henceforth, analysis of autosomes can be performed for individual identification, sex chromosomes for gender determination and mtDNA for maternal lineage.[6] As differential DNA analysis, the Y chromosome determination is an expanding routine procedure in cases of sexual abuse.[7] This technique allows segregation of male and female DNA and hence is of great value in establishing the identity of crime accused.[8] In entire human genome, there are around 30,000–40,000 known genes that code for their respective protein synthesis. These coding regions are called 'exons', and the non-coding regions in between the genes are called 'introns'.

DNA profiling

Basically, the sequencing of a person's DNA is known as DNA typing or DNA Profiling.[5] Because of the uniqueness of the DNA pattern, the sequencing is also known as DNA fingerprinting.[5,8] DNA profiling is constantly used for the spotting of both sufferers and assailants in heinous acts, such as rape, sexual assault, homicide and fire accidents. It is also used for solving cases related to maternal and paternal disputes and in wild-life forensics cases like poaching of endangered species. The technique involves collection of clean samples from the query subject and the control known sources.[9] The samples are in most of the time decomposed, adulterated, and of various unknown origins and discriminatory power of DNA profiling becomes very high, with an expected likelihood of a match between two disconnected people is estimated about 10^{-10}–10^{-13}.[5] However, issues like DNA degradation or contamination and closeness of the profiles of the victim and assailant (e.g. siblings) are unlikely to be acknowledged in courtroom especially when the other corroborative evidences are not produced.[8-10]

The Lineage Markers: mitochondrial DNA (mtDNA) and Y chromosomes

The mitochondrial DNA (mtDNA) is small, circular double helix of DNA with 16569 base pairs located in the mitochondria in the cellular cytoplasm. The mtDNA is acquired only from the mother as in the sperms, the mitochondria are present only in the mid and tail portion and only the head of sperm is involved in the fertilization. Hence the mtDNA don't pass on to the siblings from the father. Therefore, all siblings of the same mother owe the same mitochondrial genome. The nucleotides in the mtDNA code for 37 genes that in turn code for the production of two ribosomal RNAs, 22 transfer

RNAs and 13 proteins. All these 13 protein products are constituents of the enzyme complexes of the oxidative phosphorylation system. Forensically, mtDNA is most frequently used when the quantity and quality of the nuclear DNA are compromised. The mtDNA are preferred during investigations because of presence of more copies of mtDNA in a cell than the nuclear DNA.[5] Hence, the structure and the genetic constitution of mtDNA are highly preserved among mammals.[6,11] However, there is limited discrimination capacity of mtDNA when the individuals are related maternally.

Certain genes are present exclusively on the X chromosome and are referred as 'sex-linked genes'. Females have two copies of the X chromosome, and men have only one X, will get only one copy (recessive). Hence, a female must get two damaged copies of the gene in order to have a defect.[7]

DNA polymorphisms

This intergenic region in the human genome contains repetitive sequence with high amount of diversity called polymorphic regions. This may be either a 'sequence polymorphism' or 'length polymorphism'. These areas comprise DNA polymorphisms which could be utilized for forensic study and genetic mapping. The two varieties of repetitive sequences in the DNA correspond to interspersed repeats (random repeats) and tandem repeats. The tandem repeats further are of two types, namely variable number tandem repeats (VNTRs) also known as 'minisatellites' and short tandem repeats (STRs) or 'microsatellites'.[5,10] The core repeat units in VNTRs range from 10 to 100 base pairs (bp.) and for STRs range from 2 to 6 base pairs. VNTRs and STRs both are a valuable tool in DNA determination, but since genome has more STRs, their characterization becomes less cumbersome in comparison to VNTRs. The loci which are nothing but repeat sequences, range differently between individuals, and this forms the basis for genetically determining the identity of an individual and to establish his/her genetic profile. When the number of tandem repeat units differs, it is known as length polymorphism. A change in the single base pair in the sequence is known as single-nucleotide polymorphism (SNPs) or point mutation.

Technologies for DNA forensics

The conceptualization and characterization of DNA as a double helix in 1953 by James Watson and Francis Crick not only provided an explanation of its properties but also paved way for the discoveries of the application of DNA technologies.[12] A boon for the technological advancements in forensic DNA determination was the discovery of polymerase chain reaction (PCR) by Kary Mullis in 1986. The first application of DNA technology in forensic case was after the invention of DNA fingerprinting by Alec Jeffrey in 1986. The extraction of the DNA molecule from the biologic sample is the first procedure involved in the laboratory processing of the evidence.[13] The PCR-based analysis has the advantage of being highly sensitive and less time consuming.[14]

Forensic DNA profiling

Among the initial ways of forensic DNA profiling, the application of restriction fragment length polymorphism (RFLP) was the first.[12,15] In this technique, the restriction endonucleases enzyme is used to cleave specific sites along the DNA sequence. The resultant DNA fragments are separated as per their sizes using agarose gel electrophoresis which are then shifted on to a nitrocellulose sheet and hybridized with specific locus probes (chemiluminescent or radioactive) and are visualized as bands by autoradiography or chemiluminescence. RFLP needs huge quantity of DNA and is barely used in forensics as of today. Invention of PCR also is one of the other factors which has led to its degradation in forensics.

Autosomal STR profiling

The human genome contains 5–10% of repetitive sequences that occur in tandem. The STR is a region in the DNA which contains an array of such tandem repeats. The DNA sequence in such STR units is 2–6 base pairs, and more than 10^5 STRs exist in the human genome.[16] The number of tandem repeat units in the STR locus differs from individual to individual and thus determines the genotype for human identification [Figure 1]. The STRs are manifested in all the chromosomes including sex chromosomes, and their profiling requires only a small amount of DNA (~1 ng), the amount which is 50 times less than that required for RFLP analysis. With the application of PCR technology, the amplification of this 1 ng of DNA sample is possible to complete a successful STR profiling. Also the PCR amplification of multiple STR loci may be performed simultaneously in the same PCR tube. This technique is known as multiplexing or multiplex PCR. The commercially available STR markers are routinely used for forensic DNA profiling. For example, the 13 core STR markers developed by FBI in USA is known as CODIS (Combined DNA Index System).

The STR locus is of three types [Figure 2]:

1. Simple STR: The repeat units are of identical length and sequence units.
2. Compound STR: consists of two or more adjacent simple repeats.
3. Complex STR: have several repeat blocks of different unit length and variable intervening sequences.

The DNA is extracted from the sample, and the amplification of the STR loci is performed using fluorescent dye-labelled primers. The amplified products after few cycles of PCR are separated and detected via capillary electrophoresis. The detector senses the dyes which corresponds to each DNA fragment, and the computer generates the data and are represented as spikes known as electropherogram. The peaks correspond to each DNA fragment identified. The size of each peak is determined using size standard and allelic ladders. Each allelic ladder is resolved properly to determine the correct STR allele.

Single-Nucleotide Polymorphisms (SNPs)

The variations that occur between individuals at single base sequence at a particular point in the genome are termed as single-nucleotide polymorphism or SNPs.[16] SNPs constitute the commonest form of genetic variations in humans. It may be a result of base substitution, deletion or insertion at a single site. They occur at every 100–300 bases along the DNA strand. In the entire human genome, there are around 1.4 million SNPs identified and hence have the potential to be used as markers for forensic application [Figure 3]. The advantage of SNPs in forensic application over STR profiling is that the PCR amplification of the SNP marker may be able to withstand degraded DNA sample better and can be multiplexed to a higher level than STRS.[17] However, large number of SNPs need to be analysed for a reasonable discriminating power. Although SNPs are unlikely to replace the STRs analysis, they may be used as an additional tool in forensically challenging cases.[18]

Teeth as sources for forensic DNA

In most of the forensic cases, blood, saliva and semen are the biological fluids sent for DNA analysis. However, in severely decomposed, skeletonized or charred bodies, the teeth are the tissue of hope for the extraction of DNA. In medico-legal death investigations, the DNA source may be divided into two areas: firstly, DNA obtained from the outside the body or inside cavities the body (e.g., blood, saliva, semen, vaginal fluid, etc.), secondly, DNA from biological materials (e.g., liquid blood, bones, teeth, nails, etc.).[19] The nucleated cells in the teeth and the surrounding periodontal ligaments are the rich source of genetic information. After a thorough morphological and radiological evaluation for identification and age estimation respectively, the tooth is either crushed or sectioned at various levels in order to extract the DNA.[20] One of the most advocated methods of DNA extraction from tooth is the cryogenic grinding by sectioning of tooth at the cemento-enamel junction for a conservative approach to dental DNA.[21] Figures 4 and 5 depict the sequence of extraction of DNA from teeth. Pulverizing or pounding

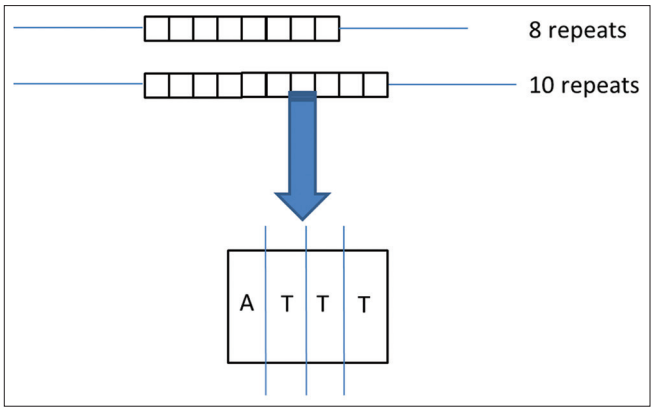

Figure 1: Schematic diagram of STR genotype. The individual has 8 and 10 repeats on the homologous pair of chromosomes. Hence, the genotype is (8,10). The repeat unit is ATTT

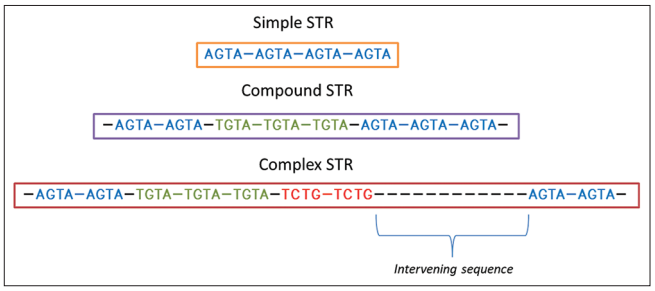

Figure 2: Schematic representation of the three types of STRs

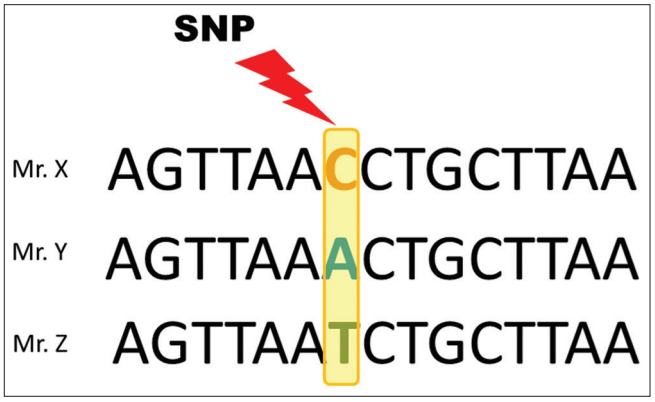

Figure 3: Schematic illustration of single-nucleotide polymorphism

the whole tooth into a fine powder can yield the sufficient amount of DNA for investigation. However, during such investigations using tooth, the recording of the other data from the tooth like the morphologic details, and the radiographical data are to be made prior to the destruction of the tooth for DNA extraction.[22]

DNA from saliva and oral mucosa in forensic investigation

Nonliving stuffs such as clothes, eating materials, cigarettes, cigars, other smoking devices, oral health devices, beverage containers, dental devices, postal stamps and envelopes can all be most readily available materials for saliva recovery.

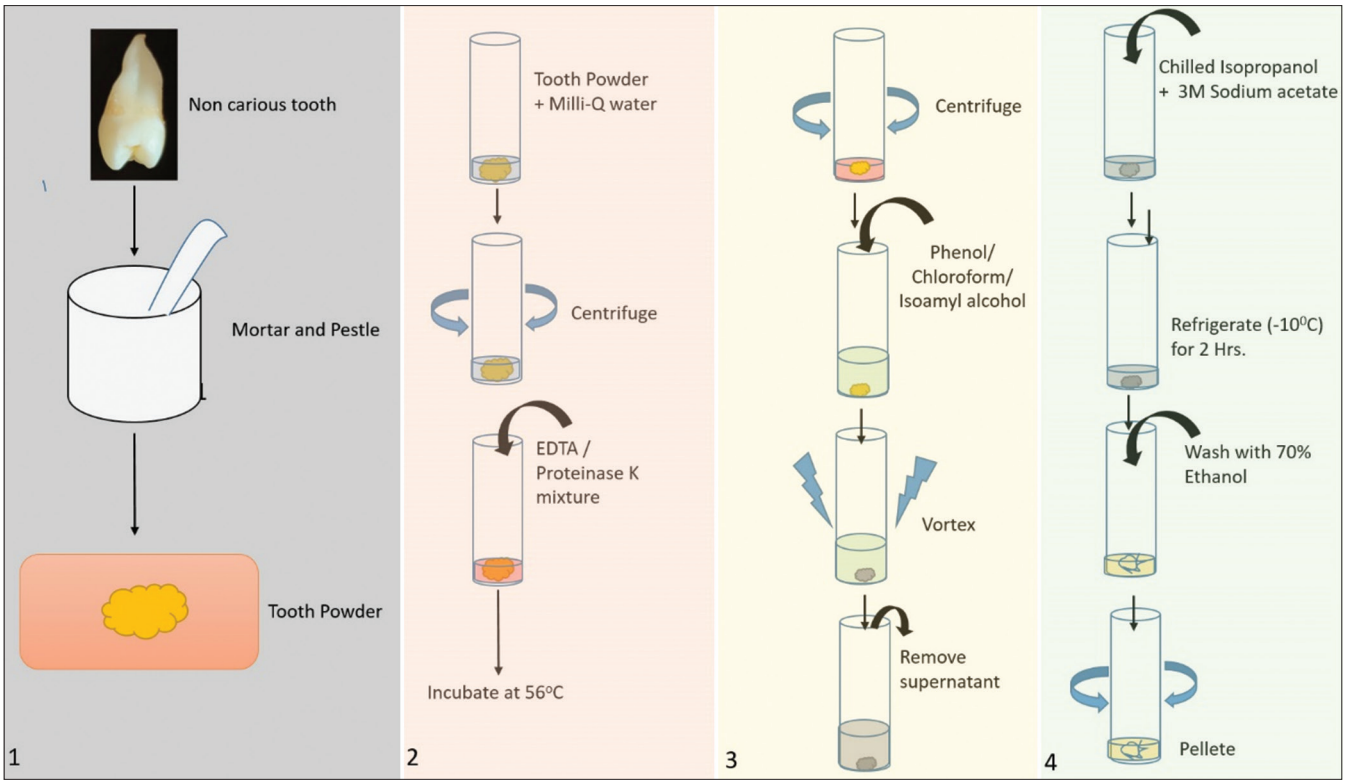

Figure 4: Schematic diagram depicting the sequence of procedures involved in DNA extraction from tooth

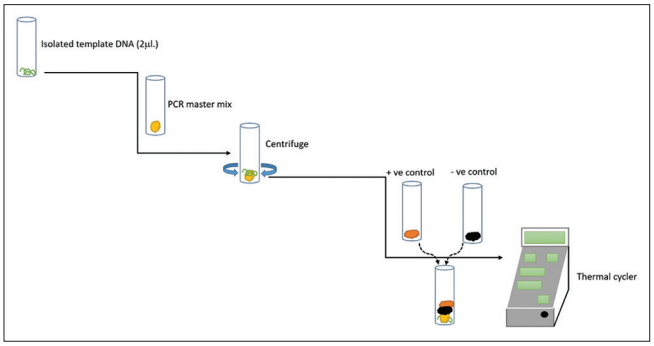

Figure 5: Schematic diagram depicting the methods of DNA analysis

Extracting salivary DNA from personal effects usually is based upon individual protocols from laboratories processing the genetic material, whenever processing a bite mark for DNA is concerned. The buccal swab technique uses collection of exfoliated oral mucosal epithelial cells for the purpose of DNA analysis. The saliva samples obtained from crime scene and the DNA analysis from the sample may help the investigation authorities link the perpetrator to the crime scene and to the victim. The buccal swab may be used as a fastidious and conservative alternative to blood for DNA sample.[23]

Salivary DNA in Bite mark evidence

In sexual assault cases involving bite marks as evidence, the visualization of the salivary stains can be enhanced using alternate light sources, leading to the preservation of DNA sample as it remains unmanipulated and also assessment can be made even in cases where a definitive injury is not suspected. Saliva in itself is devoid of DNA, but the components such as sloughed oral mucosal cells, white blood cells, mostly from the gingival crevicular fluid contain DNA.[24,25]

In 1992, a method to obtain saliva for DNA analysis was well documented, but in 1997, Sweet *et al*. gave the double swabbing for salivary DNA collection from skin which eventually became a successful method for salivary DNA collection.[26-28]

Practically, for the collection of material using buccal mucosa, a double swab technique is followed in which mouth is rinsed with water and then with a sterile gauze buccal mucosa is scraped and after that left to dry followed by scraping the mucosa twice by two different swabs and then letting the two swabs air dry after which they are placed in a labelled envelope and sent to the lab.

Washing action of rinsing leads to the washout of the probable contaminants and improves the quality of DNA hence obtained.[29]

CONCLUSIONS

With the increasing availability of DNA methods, there has been an emerging interest in the use of DNA

techniques in the forensic investigations. This has led to a revolution in the field of forensic science, including forensic odontology. The dental analysis is placed between the fingerprint analysis and DNA analysis as primary identification methods in DVI. From a forensic odontology perspective, some theoretical and practical knowledge on the DNA analysis is mandatory to the individuals involved in forensic odontology. In sexual assault cases involving bite mark, the saliva in the bite mark is sometimes overlooked and missed as a source of DNA. The stakeholders in the forensic science also are not aware of the ideal tooth for DNA extraction in most of the cases, as it varies according to the cases. Prior to the destruction of tooth for DNA extraction, the possible methods of age estimation or sex determination from the particular tooth may be attempted and recorded. The present review is an attempt to highlight the basics of DNA and DNA analysis especially from dental and saliva source to the stakeholders in legal and forensic system.

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Conflicts of interest

There are no conflicts of interest.

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