Invited Review
Journal of Forensic Dental Sciences, *Vol 12(3), DOI: 10.18311/jfds/12/3/2020.617, September-December 2020, p. 186-196*
ISSN (Print) : 0975-1475
ISSN (Online) : 0975-2137

Tooth as a Source of DNA in Forensic or Investigative Genetics: An Overview

Nandini D. B.[1]* and Juniya Grace Joji[2]

[1]Professor & Head, Department of Oral Pathology & Microbiology Dental College, Regional Institute of Medical Sciences, Imphal, Manipur, India; nanni29@rediffmail.com
[2]Independent Researcher, Mulamoottil Gilead Eraviperoor P.O. Thiruvalla, Pathanamthitta District Kerala-689542, India; juni20grace@gmail.com

Abstract

Deoxyribonucleic acid (DNA) is the genetic code of most organisms including humans. In the last few years, DNA analysis methods are applied to forensic cases for human identification which is termed *forensic or investigative genetics*. It is often challenging to obtain and interpret DNA from routine samples like blood in severely decomposed or disfigured bodies recovered from incineration, immersion, trauma, mutilation, and decomposition as in incidents of fire, explosion, or murder. The tooth can survive any extreme environment with minimum risk of contamination making it a valuable alternative source of DNA in such cases. Tooth structures like the enamel (amelogenin protein), dentin, cementum, pulp, and adherent tissues like bone and periodontal fibers are sources from which DNA can be obtained. Various methods have been described for DNA extraction. There are numerous applications of DNA analysis like identification of the deceased or missing victim or the unknown culprit from a crime scene, solving paternity issues, determining the occurrence of any genetic disease, and determining the ancestry. This article briefly summarizes an overview of the tooth as a valuable DNA source and various methods and challenges related to DNA analysis.

Keywords: Applications, DNA Fingerprinting, DNA Profiling, DNA Extraction, Forensic Genetics, Human Identification, Isolation Methods, Limitations, Lineage Markers, Short Tandem Repeat, Tooth.

Introduction

DNA is the genetic code seen in each and every cell of a human body. 99.9% of DNA sequences are similar while only 0.1% of DNA is unique and different in every person. The probability of two people other than identical twins showing similar DNA profiles is 1 in 594 trillion people[1]. Watson and Crick (1953) discovered the double-helix structure of DNA, responsible for the genetic inheritance of human beings[2]. Ever since there is a development of newer techniques that allow characterizing each person's individuality based on the DNA sequence. *DNA fingerprinting* is the process of determining an individual's DNA characteristics[3]. DNA fingerprinting is also known as *DNA profiling, DNA testing,* or *DNA typing.* The term "DNA fingerprinting" is replaced with "DNA profiling" since the analogy with fingerprints was not considered to be helpful. The process of DNA profiling was introduced by Sir Alec Jeffrey at the University of Leicester in 1985[4]. With the advancement in the field of molecular biology, DNA tests have improved and their use has offered a new perspective in human identification[5,6].

In mass disasters like earthquakes, tsunamis, fires and explosions, severely mutilated, charred, or decomposed bodies may be found and human identification will be challenging[1]. In such scenarios where there are very little remains left and morphological tests like fingerprints may not be available. Investigative or forensic genetics with DNA analysis takes up the primary role and maybe the only option for human identification in such situations[5].

*Author for correspondence

The tooth is the most durable structure in the body and can survive harsh physical and chemical environments due to its location since it is well protected by the bone and muscles. In addition, a lesser chance of being contaminated makes it an excellent DNA source[3,7,8]. The tooth DNA aids in primary identification and as an adjuvant reference sample to relate the other tissue fragments.The tooth not only serves as a good source of DNA samples but also aids in sex determination, blood group determination, and age estimation.

There are two types of DNA namely genomic DNA found in the nucleus and mitochondrial DNA (mtDNA). Genomic DNA is the most often extracted DNA type. Mitochondrial DNA is analyzed when samples are degraded or inadequate. DNA can also be classified as autosomal DNA (seen in autosomal cells) and Non-autosomal (Genomal) DNA seen in sperms and ova[9].

Sources of DNA

The sources of DNA include biological substances like blood, hair roots, nails, tissues like oral mucosa, bone, teeth and body fluids, or substances like saliva, semen, vaginal fluid, feces, and urine[10,11]. Touch DNA incorporates the use of skin cells[12]. Teeth and bone survive decomposition and autolysis as well as environmental insults, such as water immersion, burial, and fires as hot as 1,100°C, unlike soft tissues[13]. Teeth and bones have well-preserved DNA even after many years and act as alternative DNA sources[14]. Some authors suggest that teeth are better sources of DNA than bones[15].

Tooth has always been an excellent forensic tool and a good source of DNA[5,8,16]. DNA can be obtained from enamel (amelogenin), dentine, cementum, and pulp. Sometimes adherent periodontal ligament fibers and bone may be seen on the tooth surface. The majority of the dental tissues are usually not contaminated since they are well-protected by bone and soft tissue[10,17,18]. Dentin and pulp are good sources and provide an adequate amount of DNA[16]. The pulp with cellular structures like odontoblasts and other nucleated cells is the best source of DNA when available and has less chance to be contaminated as it is covered by dentin and enamel[18]. Reparative or tertiary dentin with cellular or soft tissue inclusion and dentin exposed due to wear with odontoblastic cell body sucked into the tubules due to hydraulic forces may serve as the DNA source. Apical and lateral canals in the root dentin and furcation area are also suggested for DNA retrieval. Cementocytes within the cellular cementum can also provide DNA[10,11]. The pulp is said to show the strongest DNA amplification, while dentin and cementum show similar results[20]. The dental pulp harbors the blood group antigens which is useful in forensic analysis[21]. DNA can be isolated from the crown, root body, and root apex. The root body yields the highest quantities of DNA[22]. Sex determination can be also done by using a tooth AMEL gene in enamel[23]. Age can be determined by estimating the telomere shortening of the chromosome from a tooth DNA[24,25].

Sometimes, the identification of the perpetrator is unknown. In case of unknown bite marks in human remains, DNA can be analyzed to exclude animal bites from human bites since DNA shows species differentiation. Saliva obtained from these bite marks can provide sufficient DNA if isolated immediately[8]. A "Surrogate" DNA sample is a sample thought to possess DNA from the missing person such as microscope slides, deep-frozen tissue specimens, and neonatal blood cards that have all been utilized in the past[9]. These act as adjuvants for the comparison of DNA samples retrieved. For example, toothbrushes, razors, underwear, or hairbrush, saliva beneath stamps, bedding, handkerchief, and cigarette butts. Biosensors to determine the biological origin of samples and screening for DNA integrity are based on mass spectrometry and Fourier transform infrared spectroscopy.

DNA Isolation Methods

Canine and molars are the most common teeth used for DNA isolation[18]. Some authors have obtained 6 to 50 µg of DNA from a single tooth[16,26,27]. Chowdary *et al.*, obtained 70.217 µg/ml of DNA from a freshly extracted tooth using standard conditions of extraction[23]. A high quantity of DNA can be extracted from the root portion of the tooth. Before DNA extraction, the extracted teeth may be sterilized with 0.5% sodium hypochlorite, washed with distilled water for five minutes, allowed to dry at room temperature, and subjected to ultraviolet (UV) light irradiation for one hour[28].

DNA isolation involves three stages namely cell lysis by membrane rupture, protein denaturation or inactivation by chelating substances or proteases, and lastly DNA isolation[14]. The DNA isolation techniques

include organic extraction, ion exchange, solid-phase extraction, and laser capture micro dissection. Yukseloglu *et al.*,[28] have listed some methods namely 1) Phenol-chloroform organic extraction Method 2) CTAB+Isoamyl alcohol-chloroform organic extraction method 3) Sodium chloride-sodium acetate extraction method 4) Silica extraction method 5) Chelex-100 inorganic extraction method 6) QIAamp DNA mini-kit inorganic silica-based extraction method 7) QIAamp DNA mini-kit+phenol-chloroform modified compound method 8) Qiaquick based inorganic DNA extraction method (Bosnian Method) 9) Invisorb spin forensic kit inorganic based extraction method 10) DNA IQ System inorganic based extraction method. Three different extraction methods (phenol/chloroform (organic) extraction; ammonia acetate/isopropanol and silica) have been suggested by Remualdo *et al*[29]. At temperatures as high as 500°C and 600°C, the isopropanol/ammonia acetate extraction method provides better results, for mtDNA extraction[29].

The phenol-chloroform isoamyl alcohol method combined with the QIAamp DNA mini kit methodis the most effective and successful method of high molecular weight DNA extraction[28]. Limitations of phenol-chloroform isopropyl alcohol are it takes a longer time, uses toxic solvents, is expensive, and requires multiple tube transfers involving a high chance of contamination and mislabeling[30]. Use of chelating resins with ion-exchange-approach like Chelex method is also in use[31]. Chelex 100™ (Medox Bio) and Qiagen DNA Mini Kit provide a much faster result, are inexpensive, does not contain more stages, recovers more DNA, and use non-toxic substances compared to organic techniques but obtaining pure DNA is difficult[28]. Modified Chelex technique recovers a higher quantity of DNA compared to classical Chelex method but is time-consuming. Thus it is used in deficient samples with minimal DNA content[19,32,33].

Wyman and White in 1980 observed the first polymorphic locus in the human genome using a DNA probe. Based on different sizes, loci were nominated as a variable number of tandem repeat (VNTR) or long tandem repeat or mini satellites with 9 to 80 base pairs, and Short Tandem Repeats (STR) or micro satellites with 2 to 7 base pairs. These repeated sequences spread across the human genome and were unique to assist in identification. The DNA polymorphisms do not alter during the lifetime of a person. Only identical twins share the same DNA[2,34].

Various methods of DNA profiling are:

Restriction Fragment Length Polymorphism (RFLP) Analysis

It was the first method used for DNA analysis. In this method, the collected DNA sample from the tooth is subjected to restriction enzymes named restriction endonucleases, which cut the DNA at a specific location called a restriction endonuclease recognition site[3]. Then the DNA cut-segments are separated according to their size through a process known as Gel Electrophoresis. Then the DNA pattern in the gel is transferred to a nylon membrane, a process known as Southern Blotting. Prepared DNA probes are added and excess is washed off[35]. The membrane is exposed to X-ray source. The X-ray film is developed to make visible the pattern of bands referred to as autoradiography[3,35]. This method requires that large amounts (>100 ng) of DNA thereby cannot be applied on degraded samples and takes several weeks to perform. So results may be delayed[14,30]. The cut-fragments contain VNTR sequences of intermediate size (15-65 base pairs). This method is not presently used since DNA obtained is not of optimal quality[13,36].

Polymerase Chain Reaction (PCR)

This was introduced by Kary Mullis in 1983[23]. This method allows the amplification of the DNA sequence of interest to reproduce millions of copies. In a sample test tube the DNA sample, primers, Taq polymerase enzyme, nucleotides are added and made to undergo a thermal cycle. A cycle event includes denaturing, annealing and extension[21]. This process is quicker, can produce a million-fold amplification of the desired region in 2 hours, and has improved sensitivity, speed, and genotyping precision. It is less labor-intensive and less tedious than RFLP and requires a very less amount of DNA (<100 pg)[35,37]. The limitations of PCR are that it is expensive, requires costly equipment and reagents, is technique sensitive, and there is a risk of contamination from extraneous DNA, and cross-contamination between samples leading to false-positive results[38].

Dot/Blots

This method utilizes DNA probes. "DNA probe is a tiny piece of single-stranded DNA (oligonucleotide) which will bind to another single-stranded DNA with the complementary sequence. A Sequence-Specific Oligonucleotide (SSO) probe, also known as an Allele-

Specific Oligonucleotide (ASO) probe, is a single-stranded DNA fragment sufficiently long to confer specificity, but short enough to bind only to the exact sequence complement". Commercial kits, i.e., DQ-alpha and PolyMarker systems, are based upon a dot/blot format for SSO typing and are currently in use by many crime labs. This dot/blot-strip shows a series of spots that show blue color if the result is positive and thus provides positive and negative results. This method is quite rapid and works reasonably well despite sample degradation, but does not harbor the same discriminatory power as RFLP tests[38].

Amplified Fragment Length Polymorphism (AmpFLP) Analysis

AmpFLP is a quicker method compared to RFLP analysis. It is highly-automated, and permits the creation of phylogenetic trees based on comparing individual samples of DNA with ease. It is also cost-effective and easy to setup and operates. Thus, AmpFLP is mostly used in lower-income countries. It uses PCR for DNA amplification[39].

Short Tandem Repeats (STR)

These are tiny sequences of DNA of 2 to 7 repeating base pairs repeated at multiple points in an individual's genome. They are inherited from both parents. STR are highly polymorphic and no two persons have the same STR except twins. This uniqueness of the STR makes them the biomarker of identity[14]. From country to country, different STR-based DNA-profiling systems are in use and most often used in the identification of mass disaster victims and old remains. Teeth and dense corticated bone such as femur are often the common sources for samples. Previously silver-stained polyacrylamide gels were used while now fluorescent methods with capillary/gel electrophoresis are used[14]. FBI uses a Combined DNA index system (CODIS) based on STR to create a DNA database both familial and criminal/Interpol DNA Database which have their benefits and risks. Two persons having a similar 13 loci DNA profiles is one in a billion. "The European standard set of 12 STR markers[40] and the US CODIS standard of 13 markers[41]. Due to partial overlap, they form together with a standard of 18 STR markers in total[37]. STR method cannot be applied in case of highly degraded or low copy number DNA samples and inconsistency of a number of STR markers in different DNA databases exist.

Y-Chromosomal Analysis

It involves targeting the polymorphic regions of the Y-chromosome (Y-STR) using primers. Y-chromosome is passed to the son from his father as a lineage marker that is useful for linking a person to a family or ancestral genetic pool. Gill discovered and employed Low Copy Number DNA profiling (less than 200 picograms of DNA found in a sample) in late 1990. This method is most often used to get information on human evolution, migration, paternity analysis, and sexual assaults. The lineage markers also offer the potential for a reference source that spans many generations. The disadvantages of this method are it provides only paternal information, there is contamination risk, amplification of contaminants, mixed profiles being produced, and wrongful accusations. "The largest forensic Y-chromosome haplotype database is the YHRD (www.yhrd.org) hosted at the Institute of Legal Medicine and Forensic Sciences in Berlin, Germany, with about 115,000 haplotypes sampled in 850 populations"[42].

X-Chromosomal STR

This is used in identification and genome studies of ethnic groups and as an adjuvant system in deficient paternity testing. In this method amplification is easy and shows high sensitivity[43,44].

Mitochondrial DNA Analysis

A cell contains only one copy of nuclear DNA, but thousands of copies of the 16,000-base pair mitochondrial DNA (mtDNA) sequence; hence a mitochondrial DNA type can be obtained when the nuclear DNA type cannot[38]. MtDNA is inherited strictly from mother to child with no paternal contribution. Mitochondrial DNA possesses a high copy number and a high degree of sequence variation. This method is used for mtDNA profile comparison of unidentified old skeletonized remains with highly degraded DNA with maternal relatives[38]. The method has some limitations like it is very expensive since it involves direct sequencing of its nitrogenous bases, time-consuming, requires specialized technology and provides limited information of only maternal inheritance[41]. The largest forensic mitochondrial DNA databank is EMPOP (www.empop.org) present at the Institute of Legal Medicine in Innsbruck, Austria, with approximately 3,000 haplotypes samples from 63 countries[45].

Single Nucleotide Polymorphism (SNPs) Analysis

These are variations in the DNA sequence that occur when a single nucleotide (A, T, C, or G) is altered in the genome. Eg: A**A**GGCTAA to A**T**GGCTAA. They are increasingly explored due to their small amplicon size, which assists in analyzing degraded DNA samples, and have a lower mutation rate when compared to STR. They can provide information on ancestry, lineage, evolution, identification, and gender. Their limitation is that they require larger multiplex arrays, are not suitable for DNA mixtures, have no widely established core loci, and are less informative per locus than STR[46].

Gender Typing

The AMEL gene, coding for a highly conserved enamel protein, is located on the X (Xp22.1 - Xp22.3) and the Y chromosomes (Yp 11.2. 31.32). In humans, females (XX) have two identical AMEL genes but males (XY) have two non-identical genes and both show varied lengths -106 and 112 base pairs (bp) in length respectively. This variation in size and pattern of the nucleotide sequence in the enamel of both sexes is useful for gender identification[23,47,48]. The reliability of this method is debated due to allele dropout of the X-AMEL gene in males due to the polymorphism of primer binding sites. The presence of higher variations and deletions in the Y-chromosome can affect the Yp11.2 locus. This may result in males wrongly being identified as females because of similarity in the AMEL profile. The ideal standard for sex assessment is YSTR and SRY (sex-determining regions of Y-chromosome) methods in conjunction with the AMEL testing[49]. The gender may also be identified from the dental pulp DNA by analyzing peaks of X and Y loci by capillary gel electrophoresis[26].

DNA Methylation and Microarray Technique

Methods utilizing mRNA, microRNA, immune-based assays, and DNA methylation are being explored presently. This method is applied for the identification of body fluid. It shows high specificity and compatibility with present STR testing protocols. The assay uses a set panel of loci that are differentially methylated between tissues to determine the most probable tissue source of an unknown DNA sample. Typically, approximately 1ng of DNA from the unknown sample will be digested with the HhaI methylation-sensitive restriction enzyme, which cleaves DNA at its recognition sequence GCGC only if it is unmethylated. Locus having a high methylation level is amplified more effectively, with subsequent stronger signals in the electropherogram. Conversely, loci with a lower methylation level are amplified poorly, resulting in a relatively weak signal. More definitive tissue identification can then be made by using the methylation ratio values, which are generally tissue-specific. The limitation of this method is it is labor intensive and time-consuming[50].

Microfluidic Systems for DNA Analysis

Microfluidic devices are gaining attention after the invention of the "Lab-on-a-Chip". This system has two or more micro devices or chips that can perform a single processing step, such as micro capillary electrophoresis. The micro sizes utilize minimal reagent and sample volume. Chances of contamination are minimal since the system is sealed after sample addition. Furthermore, constructing the right type of valve, mixer, and/or pump in these devices is challenging. Robotics is also being incorporated into microfluidic systems. The automated DNA extraction platform utilizing magnetic particles is a robust and more cost-effective method of extracting DNA with less chance of contamination. This method cannot be employed on samples with reduced DNA content[51].

Nanotechnology for DNA Analysis

The incorporation of nanoparticles in PCR amplification to create physical and chemical properties on the surface is also explored. For example, gold nanoparticles, carbon nanotubes, nanometer-sized polymers, and silver nanoparticles increase the PCR specificity. The actual mechanism is being investigated[52]. DNA biosensors based on nanoparticles are also being explored.

Next-generation Sequencing Technologies

Haloplex target enrichment system analyses a large number of markers like 10 STRs, 386 autosomal ancestry and phenotype informative SNPs, and the complete mtDNA simultaneously. Limitations include high error rates, less accuracy, and reliability[53].

Methods to extract the DNA from the tooth

- Crushing tooth with mortar and pestle.

- Cryogenic Grinding of tooth: It was pioneered by Sweet and Hildebrand in 1998[54]. In this procedure, liquid nitrogen is used to cool the tooth sample. The advantage of this method is it protects the DNA from heat degradation and also makes the tooth brittle. The tooth is then powdered to increase the surface area and subjected to various chemical agents to release the DNA. This method allows the recovery of DNA from remnants of pulp cells as well as the cells embedded in the hard tissues[3,8,21,55].
- Vertical sectioning of tooth: This method involves splitting through the longitudinal axis of the tooth allowing access to the entire pulp length.
- Horizontal sectioning of tooth: This method involves splitting through the cervical portion of the tooth using a high-speed rotary hand piece cooled with distilled water[21]. A brief decalcification step with Ethylenediamine-Tetraacetic Acid (EDTA) is required to get cellular samples within the hard tissues.
- Conventional Endodontic accesses: In a vital tooth, access opening can be done and the pulp can be extirpated.
- Orthograde entrance technique: Using this method Alakoç *et al*., got 80.1% of the nuclear DNA amplification products from ancient teeth with minimal damage to tooth morphology[56].

Applications of DNA Analysis

DNA profiling has many applications likehuman identification, gender determination, ethnic identification, biogeographic ancestry, immigration testing, mass disaster victim, paternity testing, pedigree analysis or ancestral lineage, monozygotic twin identification, determining the occurrence of genetic and inherited diseases, and development of cures for inherited disorders[7]. Human identification is essential for family members from an emotional and grieving concern[7]. DNA profiling has solved certain cases in India as reported by Balla[57] and Gupta *et al*[58].

Challenges in DNA profiling

After death, DNA undergoes progressive fragmentation by autolytic and bacterial enzymes, especially DNAases. Further obtaining DNA from remains is challenging and is influenced by numerous factors like status of the remains; the time elapsed from death, sample collection, storage, transport, contamination, extraction procedures, the expertise of the personnel involved, and environmental factors. Determination of utilizable DNA is a challenge. Further, availability or ease of access for DNA testing is not available in all cities, especially in India.

Whenever DNA analysis cannot be done immediately, samples must be stored. Tissues or biological fluids should not be discarded as inadequate without first attempting DNA testing and even after testing as it may be needed to be reanalyzed at a later time. Samples can be frozen or made cold to reduce autolysis (although repeated freezing and thawing are not good). Tissue supposedly well-preserved in formalin solution is mostly refractory to PCR analysis and do pose significant technical challenges[38]. Dehydration or desiccation via spray drying, spray freeze drying, air drying, or lyophilization is also suggested in case of bloodstains and bone specimens. Additives, such as trehalose (a disaccharide), or sample matrix can also be added to a dry DNA sample. Compared to DNA stored in frozen liquid, the dry medium has a higher DNA recovery in long-term storage testing[59].

Before DNA extraction, extracted teeth are treated using sodium hypochlorite to decontaminate and remove soft tissue remnants or exogenous DNA which might influence the DNA content. Compact bones and intact teeth without any disease or endodontic treatment are preferred DNA sources due to minimum or no contamination. Further, there exists a possibility of contamination by a non-native DNA source while collection, storage, and transportation. To ensure quality assurance and validation certain protocols called the "Guidelines for forensic science laboratories" have been issued by the International Laboratory Accreditation Cooperation (ILAC)[31,60]. In fresh cadavers, uncoagulated blood is the preferred source of DNA. Brain specimen is preferred in the intermediate post-mortem periodtime. DNA from mineralised tissues like bone and teeth are quite stable in advanced decomposition/putrefaction of bodies[38].

Teeth are the hardest tissue in the human body and a better source of DNA than skeletal bones[38]. DNA in the bone can be damaged due to excessive heat[31]. Ancient DNA extraction methods are quite challenging since most cell structures in tissues are not preserved or may show chemical modifications, making it difficult to get the DNA into an aqueous solution. Although various aggressive isolation methods using high temperature or strong

reagents increase DNA release, they decrease overall DNA yield by further damage to already compromised ancient DNA. Ancient bones and teeth often contain large amounts of PCR inhibitors which are responsible for interference in DNA amplification and are co-purified with ancient DNA[61].

Sources for DNA sampling may differ based on the status of the remains and time elapsed from death and environmental factors (Table 1)[9]. Environmental factors include exposure to fire (thermal insult) or corrosive substances like lime or acid (chemical insult), physical forces (such as the violent impacts encountered in transport accidents, explosions, or mechanical wave action). Body remains may be mutilated by terrestrial and marine scavengers or predators or maybe disarticulated on purpose by perpetrator to dispose of/conceal the body identity. Extreme cold weather may preserve the remains while a hot dry climate might lead to mummification. On the contrary, warm-damp status or water-immersion enhances decomposition. By contrast, an aquatic but cold and/or anaerobic environment can sometimes serve to preserve tissue[38].

The DNA retrieved should be of adequate quantity and good quality for its utilization as forensic evidence. DNA gets degraded due to various factors like temperature, sunlight, ultraviolet light, humidity leading to microbial growth, and various chemicals[13,62]. Schwartz *et al.*, studied tooth DNA status in different pH (3.7 and 10.0), temperature (4°C, 25°C, 37°C and), humidity (20, 66 and 98%), different burial (sand, potting soil, garden soil, submersion in water and burying outdoors) and periods of inhumation (1week to 6 months) and found no environmental influence on the DNA content or extraction from the pulp[13]. On the contrary, Chowdhary *et al.*,[23] showed that 26.19µg/ml and 13.995µg/ml of tooth DNA could be obtained from dental pulp when teeth were subjected to 150°C and 250°C respectively but DNA could not be retrieved at 350°C.

DNA retrieval from teeth might vary in quantity and quality based on various factors

- Type of teeth- Incisors, canines, premolars, and molars may be used. Single-rooted anterior teeth are often lost in insults while multi-rooted posterior teeth are well-preserved by compact bone and serve as specimens. Canine and molars are the most commonly used teeth for DNA isolation[7] Molars are preferred due to the greater amount of pulp volume; nearly 15-20mg of DNA can be retrieved[13].
- Condition of teeth: Intact teeth may not always be available and may show caries, periodontal disease, trauma or fracture, endodontic treatment or restoration, prostheses such as crowns, bridges, and dentures. Trauma or fractured teeth may be contaminated by external DNA. Carious teeth and periodontal disease may possess microbial contaminants. Root canal-filled teeth may not provide pulp tissue but can provide hard tissues for DNA analysis. Healthy teeth provide better DNA content both in quality and quantity than diseased teeth[10,11,63]. Some remains were identified by dental prostheses like Adolf Hitler, and Rajiv Gandhi[57].
- Time period from tooth extraction to DNA isolation: It is also important because viable pulp samples may

Table 1. Preferred DNA sources from human remains in various states[9]

Status of remains	Desirable sources of DNA
Remains displaying relatively few signs of decomposition	Blood and soft tissues are rich sources.
Remains exhibiting partial decomposition	No blood will be available and the superficial soft tissues show putrefaction. Recovering deeper tissues like muscle or bone marrow as a source of DNA is often more successful than superficial tissues since the latter samples will require additional treatment to release the DNA and purify it from the cell debris and other decomposition products.
Remains in an advanced state of decomposition	Most of the soft tissues will have lost their integrity. Remnants of bone marrow may still be present. Obtaining DNA from liquefied tissues or adipocere is rarely successful. If profiling from the putrefied soft tissues and marrow is unsuccessful, then DNA recovery should be made from skeletal structures.
Remains that are fully skeletonized (including mummified or desiccated remains)	The DNA samples are obtained from bone, tooth, hair, and nail. The preferred starting material is either a molar tooth (preferably free from an amalgam filling) or approximately 1g of compact (noncancellous) bone.

be isolated from freshly extracted teeth rather than samples stored in formalin. Teeth desiccated for one month at room temperature showed a 50% reduction in the quantity of DNA compared to fresh teeth. DNA can be retrieved from teeth upto 90 days of desiccation but with lesser value[23].

- Type of tooth structure: Variable quantity of DNA is seen in various dental tissues and DNA retrieval is affected by disease status[10,11]. Dentin and pulp DNA is more affected than cemental DNA by the disease process[10,11]. Pulp is the most preferred source for DNA as it yielded a good quantity and highest amplification of DNA followed by dentin and cementum which were identical[20,64]. Further pulp volume decreases with age rendering lesser tissue for DNA analysis in older individuals compared to young individuals. Further, diseased teeth are more often seen in the elderly[10,11]. Aggressive treatment is required to obtain adequate DNA from dentin, unlike pulp[65]. Pulp and dentin showed 100% for DNA extraction, and 85% showed positive results for blood grouping with the PCR method[66]. Well-preserved unerupted 3rd molar provided 1.35 µg of pulp DNA[16]. Retrieval of DNA from dentin and cementum requires decalcification step. If decalcification time is prolonged for days, DNA may be lost[20]. In teeth not affected by dental disease, dentine provides a better source of nuclear DNA than cementum[10,11].
- Portion or region of the tooth: The tooth shows topographic variation in both DNA quantity and quality. Some believe that the mid portion of the root is best while others say the apical portion is the preferred choice for DNA sampling[15]. The choice of area will vary from tooth to tooth[10,11].
- Method of isolation: Different methods also affect DNA isolation as described earlier. For example crushing, grinding may damage pulp tissue. Non-destructive isolation methods provide greater and more useful DNA samples[10,11].

Furthermore, social, legal, and ethical concerns are associated with DNA profiling. The reliability and robustness of DNA testing is still questioned by some individuals. Possible errors may occur during DNA analysis from the collection, sample storage, to extraction with the subsequent conviction of a wrong person[31]. Also, paternal lineage markers (Y-STRs) and maternal lineage markers (mtDNA) while not unique to the individual may still provide inclusionary or exclusionary results. PCR and DNA microarrays are expensive and require costly equipment that is simply adding to a financial burden. DNA testing facilities are not available in most places for routine use. Despite these concerns, DNA tests are largely reliable and are regarded as legal evidence in the court of law.

Forensic DNA Databases

DNA intelligence databases also provide a repository of valuable information on criminals for identification. It remains a challenge to prevent unauthorized use of these DNA profiles. There exists controversy on ethical consideration of such database on two issues[67]. First is related to an individual's constitutional rights of privacy and freedom from self-incrimination and respect for the body threatened and the second is by increasing the identification of criminals, chances that an innocent being punished is high. China has the largest offender database in the world[68]. Joy *et al* have compiled databases of various countries[69]. In India, DNA database proposal is debated over individual privacy and confidentiality issues and is yet to be established[70,71].

Conclusion

DNA profiling is essential when traditional forensic methods of human identification are not available. In severely mutilated or decomposed remains soft tissue may not be available for sampling. In such cases, bone and teeth are useful alternative sources for DNA isolation. Teeth being protected by bone and soft tissue offer better preservation and are readily available DNA sources. Various DNA methods are available with their advantages and disadvantages. Obtaining a DNA sample of an adequate quantity and quality remains a challenge due to various factors as discussed. Developing methods which are less variable, cost-effective, and more reliable is essential. By far, DNA analysis at present is more reliable and less controversial in the courts of law. Currently, research is focused on developing methods that yield good DNA from degraded and compromised samples. Teeth serve as an important DNA source in forensic or investigative genetics.

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How to cite this article: Nandini D. B. and Joji, J. C. Tooth as a Source of DNA in Forensic or Investigative Genetics: An Overview. J Forensic Dent Sci 2020;12(3):186-196.

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