

Content available at: <https://www.ipinnovative.com/open-access-journals>

Indian Journal of Forensic and Community Medicine

Journal homepage: <https://www.ijfcm.org/>**Review Article****DNA in forensic odontology: An overview****Rubeena Anjum<sup>1</sup>, Mandeep Kaur<sup>1</sup>, Mohd Hussain<sup>1,\*</sup>, Shugufta Shafi<sup>1</sup>**<sup>1</sup>Dept. of Oral & Maxillofacial Pathology & Oral Microbiology, Indira Gandhi Govt. Dental College, Jammu and Kashmir, India**ARTICLE INFO***Article history:*

Received 08-11-2021

Accepted 07-03-2022

Available online 08-03-2022

*Keywords:*

Forensic odontology

DNA profile

Human identification

STRs typing

Cryogenic grinding

**ABSTRACT**

Human remains are tremendously damaged and degraded in forensic cases and the identification becomes very difficult, in those cases teeth and bones are often the only reliable sources of DNA for identification. Advances in DNA extraction techniques and short-amplicon DNA typing have greatly increased our potential to identify human remains which were previously considered to be too compromised for genetic analysis. As the teeth are largely protected within the jawbones and remains protected from the environmental conditions and prevent these tissues from postmortem decomposition and DNA decay. DNA profile tests which are performed nowadays are totally reliable and give details about an individual's physical characteristics, ethnicity, place of origin and sex. These tests are also accepted as legal proofs in courts. These tests are: Restriction Fragment Length Polymorphism Typing, STRs Typing, Mitochondrial DNA Analysis, Y-Chromosome Analysis, X-Chromosome STR, Single Nucleotide Polymorphism. The aim of this review is to provide a better understanding and knowledge about the latest techniques in DNA for human identification in the field of forensic odontology.

This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: [reprint@ipinnovative.com](mailto:reprint@ipinnovative.com)

**1. Introduction**

The importance of Forensic Dentistry for human identification lies mainly when there is little remaining material to perform such identification (e.g., in fires, explosions, decomposing bodies or skeletonized bodies), has led dentists working with forensic investigators to become more aware with the new molecular biology technologies.<sup>1</sup> Mass disaster identification conventionally relies on the teamwork of different experts, such as police, forensic odontologists, physicians, and pathologists. Antemortem information from the missing persons is compared with post-mortem data of the deceased persons. Due to unavailability of such data then the precise identification of individual becomes very complicated and in such cases DNA profiling system can help in exact

identification of person. Dental tissues are resistant to environmental assaults such as incineration, immersion, trauma, mutilation and decomposition and due to which dental tissues represent an excellent source of DNA material.<sup>2</sup> The DNA profile tests are reliable which are being accepted as legal proofs in courts for the investigation of paternity and human identification. Several biological materials like bone tissue, hair bulb, biopsy sample, saliva, blood and other body tissues may be used for separation of DNA and accomplishment of laboratory tests for human identification. Advances in DNA extraction techniques and short-amplicon DNA typing have greatly increased our potential to identify human remains which were previously considered to be too compromised for genetic analysis. Currently short tandem repeat (STR) typing of nuclear DNA is the most frequently relied upon technique available for human identification.<sup>3</sup> However, recent advances in

\* Corresponding author.

E-mail address: [hussynmazhar01@gmail.com](mailto:hussynmazhar01@gmail.com) (M. Hussain).

single nucleotide polymorphism (SNP) and insertion and deletion (indel) typing provide methods that can augment STR typing in cases where the DNA is highly degraded and also provide ancestry and/or phenotypic information when there is no presumptive identification or comparative ante-mortem sample available. When analysing samples containing little or no nuclear DNA, mitochondrial DNA (mtDNA) analysis may be useful. Teeth are a preferred skeletal source of DNA due to their unique composition and location within the jawbones and are largely protected from the environmental and physical conditions that act to accelerate the processes of post-mortem decomposition and DNA decay. Therefore, DNA extracted from teeth is often of higher quality and is less prone to contamination than DNA extracted from bones.<sup>3</sup>

### 1.1. Guidelines for obtaining dental DNA

For obtaining a dental tissue for DNA sampling first debride the tooth of any plaque or calculus with a curette, then wash thoroughly with H<sub>2</sub>O<sub>2</sub> followed by ethanol. A conventional endodontic access and instrumentation is done for an intact tooth which is supposed to have been removed from the alveolus recently. The access opening is done and the wall of the pulp chamber can be curetted with a slow rotary burr and the pulp tissue is collected in a wide-open sterile tube. The pulp may be mummified parchment like in dried specimens. After instrumentation, the pulp chamber is best irrigated with buffer. Ultrafiltration of liquid at lab will remove the cellular material needed for analysis. Finally crushing the tooth may be required.<sup>2</sup> Various techniques are used to locate the cells that harbour potentially useful DNA:

Entire tooth is crushed and DNA is retrieved, conventional endodontic access is done, horizontal section of tooth with aggressive extirpation and apicectomy of tooth is done and horizontal section of tooth with aggressive pulpectomy and crushing of radicular half of the tooth. (Smith et al., 1993)<sup>4</sup> “Orthograde entrance” providing a direct entrance from enamel surface to pulp cavity, this technique enables researchers to obtain more pulp as well as dentin rich sample by using endodontic files with coarser ridges.<sup>5</sup>

The conditions of the material to be examined must be carefully evaluated by the researchers, in which there is a greater risk of sample contamination and influence of environmental factors, in addition to a small amount of DNA material available in most situations, which may also include PCR-inhibitors. Various environmental factors can lead to the degradation of DNA which includes time, temperature, humidity (facilitating the growth of microorganisms), light (both sunlight and UV light) and exposure to various chemical substances.<sup>6</sup>

## 1.2. DNA analysis

### 1.2.1. Restriction fragment length polymorphism

RFLP is employed for analysing the variable lengths of DNA fragments that result from digesting a DNA sample with an enzyme called “restriction endonuclease” which sections DNA at a particular sequence pattern referred to as a restriction endonuclease recognition site. RFLP cannot be performed with the samples degraded by environmental factors and also takes longer time to induce the results because it requires relatively large amounts of DNA.<sup>6</sup>

Mutation within the zone where the restriction enzyme attacks the strand, an area that is normally cut, is not recognized. The two pieces remain together forming a protracted piece that has more resistance against migration within the gel compared to the two pieces separately. Distance between two restriction enzymes alternate through insertion, deletion, or variation in a number of repeating units. This is particularly useful in forensic science.<sup>2</sup> In a study done by Y. J. Zhang et al., multiple RFLP genotypes of *Porphyromonas Gingivalis* colonised a single periodontal pocket.<sup>7</sup>

Forensic application of PCR-RFLP genotypic comparison of *Streptococcus Mutans* has been recovered from bite injuries thus playing an important role in Forensic Odontological analysis.<sup>8</sup>

### 1.3. STRs typing

These are described as short stretches of DNA that are repeated at various locations throughout the human genome and this technology is employed to gauge specific regions (loci) within nuclear DNA.<sup>6</sup>

STRs have a high power of individual discernment due to their high standards of polymorphic informative content. The non-overlapping size of the alleles from different contributors serves to differentiate them. Currently, they are detected by fluorescent detection methods using capillary or gel electrophoresis and even by ABI gel-based DNA sequencers while earlier works on detection involved silver-stained polyacrylamide gels. Utilized in paternity testing as each individual has some STRs inherited from father and a few from the mother. They are hyper-variable regions that show repetitions of fragments having 2–7 base pairs. It helps in identifying victims of mass calamities from even old remains.<sup>9</sup> In a study done by Shbair M et al., Reliability of STR markers in carious part of the teeth was evaluated in 120 carious teeth, result of which gave insights that the carious tissues of human carious teeth could be as valid as the healthy teeth for forensic human identification.<sup>10</sup>

### 1.4. Mitochondrial DNA (mtDNA) analysis

mtDNA may be a powerful tool for forensic identification because it possesses high copy number, maternal inheritance, and high degree of sequence variability.

Each offspring has the identical mtDNA as their mothers since the mitochondrion of every new embryo comes from the mother's ovum and also the nuclear DNA is contributed by father's sperm.<sup>6</sup>

High molecular weight mtDNA are obtained from teeth, especially in degraded remains. It is thus a valuable tool in identifying missing persons by comparing mtDNA of unidentified remains thereupon of a possible maternal relative. Because the technique involves direct sequencing of nitrogenous bases, therefore it is expensive. Moreover, it provides limited information due to the involvement of familial relationship which may be traced from the feminine member of the respective family.<sup>7</sup>

A study done by Ginther C et al. mtDNA was extracted from teeth stored from 3 months to 20 years. Tooth donors and or their maternal relatives provide blood or buccal cells from which mtDNA was also extracted. Enzymatic amplification and direct sequencing of roughly 650 nucleotides from 2 highly polymorphic region of mtDNA yielded identical sequence for each comparison of tooth and fresh DNA, suggesting that teeth provide an excellent source for high molecular weight mtDNA that can be valuable for extending the time during which decomposed human remains can be genetically identified.<sup>11</sup>

### 1.5. Analysis of Y chromosome

It involves targeting of the polymorphic regions of the Y chromosome (Y - STR) using primers. As Y chromosome is passed to the son from his father, analysis of markers on the chromosome helps in sketching relationships among males.<sup>7</sup>

Majority of the length of the human Y chromosome is inherited as a single block in linkage from father to male offspring as a haploid entity. Hence Y chromosomal DNA variation has been mainly used for investigations on human evolution and for forensic purposes or paternity analysis.<sup>6</sup> In a study by Tsuchimochi T et al (2002),<sup>12</sup> they used chelex method to extract DNA from the dental pulp and amplified it with PCR and typing at Y-chromosomal loci to determine the consequences of temperature on the sex determination of the teeth.<sup>13</sup> Whittaker and colleagues determined sex from necrotic pulp tissue stained by quinacrine mustard using fluorescent Y chromosome test for maleness and claimed that up to five weeks after death, sex determination can be finished with high degree of accuracy.<sup>14</sup>

Duffy et al.<sup>15</sup> have showed that Barr bodies and F bodies Y chromosomes are preserved in dehydrated pulp tissues up to one year and pulp tissues retain sex diagnostic characteristics when heated up to 100°C for 1hour.

### 1.6. X-Chromosome STR

Analysis of ChrX short tandem repeat markers (STRs) can successfully embrace the answer that unties the challenge presented in particular cases of relationship analysis, when

the offspring is female. Since fathers transmit the same X chromosome to all their daughters, they are particularly useful in deficiency paternity cases when the child is a female, in maternity testing, and in paternity cases involving blood relatives.<sup>16</sup>

X chromosome STRs, a complementary tool to autosomal STR and mitochondrial DNA (mtDNA) markers. Moreover, higher mean exclusion chance (MEC) values are obtained when using X chromosome markers in trios involving daughters.<sup>17</sup>

Since the size of X-chromosome STR alleles is small, generally including 100-350 nucleotides, it is relatively easy to be amplified and detected with high sensitivity. X-Chromosome STR markers are a powerful complimentary system especially in deficiency paternity testing.<sup>6</sup>

Hanaoka et al. (1996)<sup>18</sup> conducted a study to determine sex from blood and teeth by PCR amplification of the alphoid satellite family using amplification of X (131 bp) and Y (172 bp) specific sequences in males and Y specific sequences in females. It was showed to be a useful method in determining the sex of an individual.

## 2. Conclusion

Importance of forensic odontology become apparent in violence and crimes against human life like bomb explosions, wars or plane crashes, as well as cases of carbonized bodies or in advanced stage of decomposition, among other circumstances in which the conventional methods of identification become impractical thus highlights the need to employ ever faster and more accurate methods during the process of forensic identification. As teeth are preserved within the alveolar bone thus preserving the integrity of genetic material and become a potential source of DNA material which play an important role in identification and criminology. DNA stores the genetic material and is unique to every individual. The DNA profile tests in forensic identification provides a new perspective in forensic identification and are totally reliable. Therefore, dental professionals working on the field of Forensic Dentistry should incorporate these newer technologies in their work for the extraction of DNA from genetic materials.

## 3. Source of Funding

None.

## 4. Conflict of Interest

None.

## References

1. Silva RHD, Peres AS, Oliveira RND, Oliveira FTD, Peres SHS. Use of DNA technology in forensic dentistry. *J Appl Oral Sci.* 2007;15(3):156–61. doi:10.1590/S1678-77572007000300002.

2. Chaudhary RB, Shylaja MD, Patel A, Patel A. DNA in forensic odontology: New phase in dental analysis. *Int J Forensic Odontol.* 2020;5(1):43–50. doi:10.4103/ijfo.ijfo\_12\_20.
3. Higgins D, Jeremy J. Austin Teeth as a source of DNA for forensic identification of human remains: A Review. *Sci Justice.* 2013;53(4):433–41. doi:10.1016/j.scijus.2013.06.001.
4. Smith BC, Fisher DL, Weedn V, Warnock GR, Holland MM. A systematic approach to the sampling of dental DNA. *J Forensic Sci.* 1993;38(5):194–209.
5. Alakoc D, Aka PS. Orthograde entrance technique” to recover DNA from ancient teeth preserving the physical structure. *Forensic Sci Int.* 2009;188(1-3):96–8. doi:10.1016/j.forsciint.2009.03.020.
6. Datta P, Sood S, Rastogi P, Bhargava K, Bhargava D, Yadav M. DNA Profiling in Forensic Dentistry. *J Indian Acad Forensic Med.* 2012;34(2):156–9.
7. Zhang YJ, Yasui S, Yoshimura E, Ishikawa I. Multiple restriction fragment length polymorphism genotypes of *Porphyromonas gingivalis* in single periodontal pockets. *Oral Microbiol Immunol.* 1995;10(2):125–8. doi:10.1111/j.1399-302x.1995.tb00132.x.
8. Mcdowall I, Wood P. Restriction fragment length polymorphisms of mutans streptococci in forensic odontological analysis. *Int J Stud Res.* 2010;3(2):166–78. doi:10.1093/biohorizons/hzq021.
9. Aditi S, Akshat S, Sumit B, Geetika A. DNA Fingerprinting - An Asset to Forensic Dentistry. *Biomed J Sci Tech Res.* 2018;9(5):1–4. doi:10.26717/BJSTR.2018.09.001855.
10. Shbair M, Adnan A, Hao P, Liu Y. Assessing the viability of carious lesions in human identification using STR typing. *Sci Prog.* 2021;104(2):1–12. doi:10.1177/00368504211008054.
11. Ginther C, Tarver LI, King M. Identifying individuals by sequencing mtDNA from teeth. *Nat Genet.* 1992;2(2):135–8. doi:10.1038/ng1092-135.
12. Tsuchimochi T, Koyama H, Matoba R. Chelating resin-based extraction of DNA from dental pulp and sex determination from incinerated teeth with Y-chromosomal aliphoid repeat and short tandem repeats. *Am J Forensic Med Pathol.* 2002;23(3):268–71. doi:10.1097/00000433-200209000-00013.
13. Monali C, Pritam P, Tapan M, Kajal D. Gender Determination: A View of Forensic Odontologist. *Indian J Forensic Med Pathol.* 2011;4(4):1–5.
14. Whittaker DK, Lewelyn DR, Jones RW. Sex determination from necrotic pulpal tissue. *Bri. Dent J.* 1975;139(10):403–5. doi:10.1038/sj.bdj.4803645.
15. Duffy JB, Waterfield DJ, Skinner MC. Isolation of tooth pulp cells for sex chromatin studies in experimental dehydrated and cremated remains. *Foren Sci Int.* 1991;49(2):127–41. doi:10.1016/0379-0738(91)90073-r.
16. Hameed IH, Kareem MA, Rafid H, X. Chromosome short tandem repeat, advantages and typing technology review. *Afr J Biotechnol.* 2015;14(7):535–41. doi:10.5897/AJB2014.14115.
17. Damascena NP, Filho MVC, Souza GRBD, Silva LAFD, Estevam CS. Testing the extraction of DNA from human teeth exposed to different chemical solutions. *Int J Odontostomat.* 2017;11(2):173–7.
18. Hanaoka Y, Minaguchi K. Sex determination from blood and teeth by PCR amplification of the aliphoid satellite family. *J Foren Sci.* 1996;41(5):855–63.

### Author biography

**Rubeena Anjum**, Professor and HOD

**Mandeep Kaur**, Assistant Professor

**Mohd Hussain**, Post Graduate Student

**Shugufta Shafi**, Post Graduate Student

**Cite this article:** Anjum R, Kaur M, Hussain M, Shafi S. DNA in forensic odontology: An overview. *Indian J Forensic Community Med* 2022;9(1):25–28.