REVIEW

Molecular Analysis in Forensic Odontology: A Review

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Abstract

Introduction: A subfield of forensic sciences called forensic odontology works with identification in cases of mass disasters, sexual assault, child abuse, etc.

Material & Methods: Scientific databases were searched for the literature and relevant articles were selected for the review.

Results: In circumstances when bodily tissues are destroyed during criminal investigations or large-scale disasters, forensic odontology investigates the field of human identification using dental tissues. Dentists are also involved in dental forensics in legal and criminal matters.

Conclusion: Teeth are an excellent source of genomic DNA since they have a large amount of storage space for DNA. Because teeth have a natural resilience to chemical and physical aggression, advances in molecular science, such as DNA analysis, have expanded the scope of forensic dentistry.

Keywords: DNA, Forensic odontology, and Polymerase chain reaction.

Introduction

The area of forensic odontology studies human identification in situations when body tissues are lost during criminal investigations or large-scale natural disasters.[1] The field of forensic dentistry is defined as identifying the physical, chemical, and biological alterations that occur in the skeletons and other human parts or facts of a living or deceased person.[2] In recent years, the application of DNA analysis has expanded the scope of forensic dentistry.[3] A branch of medical science that, in the field of justice, deals with the proper handling and examination of dental evidence with the proper evaluation and presentation of the dental findings" according to Keiser & Neilson stated forensic odontology, also known as forensic dentistry, in 1970.[4]

In forensic dentistry, injuries to the jaws, teeth, and soft tissues of the mouth are examined in order to accomplish three main areas of application: bite mark assessment, person identification, and diagnostic and therapeutic purposes.[5] A wide range of body fluids, comprising blood, semen, tissues, organs, hairs, nails, saliva, and urine, may be analyzed to determine DNA.[1] Since teeth are most durable and typically withstand postmortem degradation of all the tissue in the body, they are essential for forensic identification. Because dental tissues are capable of resisting a variety of environmental stresses, including burning, submersion, trauma, mutilation, and deterioration, teeth provide a great place for discovering DNA.[6] Due to the greater availability of nucleated cells, the pulpal tissue of teeth has a high genetic information content. The resistance of teeth against assault, whether it be chemical or physical, is unique. A tissue sample, other than the tooth, represents a significant risk of contamination or DNA degradation.[1]

The establishment of DNA's double helix structure in 1953 by James Watson and Francis Crick, which accounts for human genetic inheritance, revolutionized almost every scientific discipline and brought about significant improvements.[7] It was reported that the first DNA analysis of victims of a big disaster was done during the Scandinavian Star Ferry in 1990.[8]

In forensic DNA analysis, a typical genotyping workflow

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comprises tasks including evaluating the evidence, identifying bodily fluid, extracting DNA, reviewing extracted DNA, amplifying target loci, seeing amplified products, assessing data, and generating a report.[9]

DNA PROFILING / DNA FINGERPRINTING

Professor Sir Alec Jeffreys developed DNA profiling, commonly referred to as DNA fingerprinting or DNA typing, in 1984. It serves as an approach to recognize a person's DNA characteristics.[10] Initially, Jeffrey developed a technique called restriction fragment length polymorphisms (RFLP) to generate fragments of genomic DNA by using restriction enzymes.[11]

PRINCIPLE FOR DNA PROFILING

Just 2–5% of DNA can be accounted for by the gene that codes for a certain protein; the remainder, or 95%, of DNA is comprised up of junk or noncoding DNA. This non-coding DNA can appear as repeated DNA, which is multiple copies of the DNA spacer, or as a single copy. Long strands of this repeated DNA sequence are known as STRs. The basic principle of DNA fingerprinting and DNA profiling is the change in the micro satellite pattern discovered by a probe paired with stable inheritance.[8]

Following are the DNA types indicated in DNA profiling:[10]

DNA types

Genomic DNA	**Mitochondrial DNA**
Teeth serve as a reliable source of genomic DNA. They are situated in the cell's nucleus	Utilized when a poor quality or inadequate DNA sample is received.

STEPS FOR DNA RESEARCH

DNA has a unique quality that makes it helpful for forensic work. Temperature, pH, salt, and other unfavorable parameters that typically destroy traditional serological markers can be tolerated by DNA. Following are three basic steps for DNA research:[1]

STEP 1: EXTRACTION

STEP 2: QUANTITATION

STEP 3: ANALYSIS & INTERPRETATION

DNA COLLECTION TECHNIQUES

Fig. 1: Guidelines to procure dental DNA (Stravinos et al)[12]

- Samples of soft tissue or blood adhering to the tooth should be taken.
- Using a curette, remove any plaque or calculus from the tooth and completely disinfect it with hydrogen peroxide and ethanol.
- In cases where the tooth is unrestored, non-carious, and intact, a traditional endodontic access can be carried out.
- Greater access to the pulp chamber is made possible by sectioning the tooth (vertical axis sectioning).
- After the tooth is opened, a slow-speed rotary bur can be used to curet or instrument the pulp chamber walls. After that, pulp tissue can be gathered over a sterile container with a wide mouth.
- Crushing teeth is a possible choice.

MOLECULAR TECHNIQUES IN DNA ANALYSIS

1. Restricted fragment length polymorphism: Among the initial ways of forensic DNA profiling, the application of restriction fragment length polymorphism (RFLP) was the first.[13] Using the restriction enzyme, DNA strands are broken up into fragments in this method. After being electrophoresed on an agarose gel, the broken DNA strands are transferred to a nylon membrane. Incubation with a radioactive probe made up of complementary polynucleotide fragments that can migrate and combine with fragments of DNA.[8,13] The following are the steps involved in RFLP: extraction of DNA, fragmentation, gel electrophoresis, and band visualization.[14]

2. Polymerase chain reaction (PCR): The highly sensitive molecular technique known as PCR is tremendously helpful to forensic DNA research. The advantage of this method is that it works faster and needs less effort. The risk of cross-contamination is the only procedural downside. For the PCR procedure, two materials are needed: a primer and a DNA template.[8]

The process began with the sample tissue being heated to 96°C for five minutes. This is to ensure the primers and DNA strands are melted. To start denaturation, the sample is heated for 30 seconds at 96°C. This stage is to ensure that the nucleic acids' hydrogen bonds disintegrate. Annealing is achieved by heating the sample to 68°C for 30 seconds. The single-stranded primers will delay around at this temperature because ionic bonds between the primer and the single-stranded template will continually form and break. The production of double-stranded DNA is facilitated by this stage, which helps to establish a strong bond between the template and single-stranded primer. Upon binding to the template, the polymerase enzyme can now start duplicating the DNA, resulting in a fresh copy. Elongation is achieved by treating the sample for 45 seconds at 72 °C. For the polymerase enzyme, this is the optimal operating temperature.[1,15,16]

Short tandem repeats: Genomic regions with core repeat sequences ranging from three to seven base pairs are known as short tandem repeats (STRs). Core repeat sequences of more than seven base pairs are known as long tandem repeats, or LTRs. In forensic science labs, dinucleotide repeats are rarely employed since they artificially create shadow and stutter bands.[1,17] When doing tests with STR typing, standard DNA profiling protocol is used. The STR typing process, however, is dependent upon the standard operating protocols provided by the producers of commercial kits intended for use in forensic labs.[18,19]

Mitochondrial DNA: Oxidative phosphorylation occurs in mitochondria and results in the production of cellular energy. These procedures involve proteins that are encoded from nuclear and mitochondrial DNA. We can therefore conclude

that DNA is present in both the nuclei and the mitochondria of cells.[20] There can be numerous mitochondria in a single cell, and each mitochondria can hold several DNA particles. This suggests that a cell with a single copy of nuclear DNA can live with hundreds of copies of mitochondrial DNA.In the event that nuclear DNA is unavailable, it is proposed that Mt DNA be taken into consideration for further research.[21,22]

TABLE1: Studies summary on gender determination by using molecular analysis in forensic dentistry

S.No	Reference	Sample Source	No. of samples	Technique	Result	Conclusion
1.	Manju R Nair et al. (2020)[23]	Dental pulp	120 deciduous teeth	PCR	Compared to teeth kept in fresh water, the pulp tissue of teeth preserved in natural soil displayed a noticeably greater rate of accurate interpretation.	Teeth kept in natural soil had a higher percentage of correctly determined gender by PCR technology than teeth preserved in fresh water.
2.	Lim et al. (2019)[24]	Teeth	17 extracted teeth with and without caries	Nested PCR	76.47% of samples were accurately identified with AMEL gene.	Burnt samples can be used to establish sex type. Aside from that, dental sample restricted and deteriorated genetic material can be amplified using nested PCR, a valuable approach.
3.	Chowdhury et al (2018)[25]	Dental pulp and teeth	130 premolars	PCR	Most samples had effective DNA extraction, with the exception of teeth heated to 350 degrees Celsius. In terms of sensitivity and specificity, the determination of sex was 100%.	Teeth can be used to determine sex, are resistant to extreme environments, and were a powerful source of DNA.
4.	Kholief et al (2017)[26]	Dental pulp	40 sound and carious extracted teeth	PCR	100% success rate in determining sex without any false positive or false negative results.	SYS14 & SRY genes were efficient for sex determination.
5.	Hanaa M.H. Aal-Hamdan et al (2014)[27]	Maternal plasma/ serum	30 pregnant women (in the third trimester of pregnancy)	RT-PCR	All plasma samples from pregnant women carrying male fetuses yielded full and partial Y-STR profiles, whereas all plasma samples from pregnant women carrying female fetuses yielded negative Y-STR profiles.	The viability of using Y-STR typing to find the gender of the fetus in the third trimester of pregnancy with an accuracy rate of 100% as a gender-determining test.
6.	Manisha M Khorate et al (2014)[28]	Dental Pulp	100	Fluorescent microscope	Gender determination from human pulp is possible upto 7 weeks. The percentage of FB and BB decrease gradually as the time interval increases.	The age of an individual has no bearing on the gender that may be determined from their pulp. The accurate and economical method of determining gender is based on the presence or lack of the X (BB) and Y (FB) chromosomes.
7.	Prashant M. Battepati et al (2013)[29]	Dental Pulp	30	PCR	Using PCR amplification, sex was identified by looking for alphoid centromeric repeat sequences that are unique to the X and Y chromosomes.	When it comes to stored teeth, dry teeth can be a more reliable source of DNA than moist teeth. It is quick, precise, sensitive, and dependable to use PCR for the co-amplification of X and Y specific sequences.

Discussion

Teeth - Genetic material source

Dentin and pulp can be used to successfully extract DNA. One significant benefit of using teeth for DNA research is that they can yield large amounts of high-quality DNA. DNA is a significant source of information because it is long-preserved in teeth and bones. Numerous techniques for removing DNA from teeth have been documented, including aspiration, scraping, and horizontal or vertical tooth sectioning at the cemento-enamel junction or up to the root tip. Additional techniques include traditional access cavity preparation and dental pulp removal, cryogenic grinding, or crushing of the teeth. The simplicity, affordability, and preservation of tooth integrity that can be taken into account in forensic investigations are the benefits of the access cavity preparation approach. 10

Molecular Genetics Studies

In the field of forensics today, PCR is a vital technique, particularly when it comes to amplifying victim or suspect nucleic acid sequences and then matching them in situations involving few samples. Saiki et al. were the first to demonstrate PCR, while Mullis and Faloona were the first who utilized it in DNA synthesis.31,32 Amelogenin, SRY, DXYS156, and TSPY were the molecular markers used in PCR analysis for forensic sex determination.33,34

Utilizing enzymes and concomitant primer extension of the complementary DNA strands, PCR is an in-vitro method for replicating specific DNA sequences.30 Prior to the development of heat-stable polymerases, the application of PCR was restricted. The majority of PCR-based methods make standardization easier, boost the sensitivity of the findings, and make it simple to evaluate old or severely deteriorated samples.35 To determine the quantity of male or female DNA in a mixed sample, as in sexual assault cases, real-time PCR or quantitative PCR was developed.13 Sex typing for destroyed samples was effective, and Lim et al.'s 2019 research demonstrated that genetic material could be evaluated. In addition, dental samples with limited and deteriorated genetic material can benefit from the use of nested PCR as a beneficial approach.36 Reesu et al. (2015) state that the length of time exposed to fire, the temperature at which it occurs, and the existence of materials in between teeth can all have a substantial impact on how fire affects teeth. Reesu et al. (2015) state that the length of time exposed to fire, the temperature at which it occurs, and the existence of materials in between teeth can all have a substantial impact on how fire affects teeth.24 The amount of DNA from a tooth sample varied from 6 micrograms to 50 micrograms, as demonstrated by Potsch et al. in 1991.

Short tandem repeats (STR) analysis is commonly used for recognizing hypervariable regions of DNA in forensic samples. These segments comprise repeated repetitions of fragments containing two to seven base pairs.37 The December 26, 2004, tsunami that ravaged the Indian Ocean created significant challenges for dead body forensic identification.38 In 1991, high molecular weight DNA was extracted from tooth pulp by Schwartz et al. in a variety of environmental settings.39

DNA analysis has been useful in identifying the deceased when other dental techniques have failed.38 Therefore, teeth are a great place to find DNA because they are covered with layers of muscle, bone, connective tissue, and epithelium. Studies on molecular biology as it relates to human identification will probably enhance DNA extraction even more as less material becomes available and as adverse circumstances worsen.6

Conclusion

In the realm of forensic sciences, DNA technology is well-established, and forensic odontology has developed a special interest in gathering DNA from the body's hard tissue remains, especially from teeth. Given the belief that teeth are a repository of DNA, more investigation into methods for obtaining DNA from teeth could broaden the scope of molecular advances in forensic sciences and strengthen the notion that DNA functions as "a molecular signature in forensic odontology." DNA analysis is frequently utilized in criminal investigations, family investigations, and mass disasters.

References

1. Babu Rs A, Rose D. Molecular Advancements in Forensic Odontology. West Indian Med J. 2015 May 11;65(2):369-74. doi: 10.7727/wimj.2014.109. PMID: 26645597.
2. Sinha M, Rao IA. Genetics and Molecular Biology Techniques Applications in Forensic Dentistry-A Review. Int. J. of Multidisciplinary and Current research.2017;5:315-19.
3. Jeddy N, Ravi S, Radhika T. Current trends in forensic odontology. J Forensic Dent Sci. 2017 Sep-Dec;9(3):115-19. doi: 10.4103/jfo.jfds_85_16. PMID: 29657486; PMCID: PMC5887632.
4. Kumar, Jayanth. (2016). Virtual Autopsy. International Journal of forensic odontology. 2016:14-16.
5. Menon, Aishwarya P, Kumar, Anoop N. Recent Advances in Forensic Odontology: An Overview. Journal of Forensic Science and Medicine 7(3): p 105-08, Jul–Sep 2021. DOI: 10.4103/jfsm.jfsm_41_20
6. Chaudhary RB, Shylaja MD, Patel A. DNA in forensic odontology: New phase in dental analysis. Int J Forensic Odontol. 2020; 5(1):43-47.
7. da Silva RH, Sales-Peres A, de Oliveira RN, de Oliveira FT, Sales-Peres SH. Use of DNA technology in forensic dentistry. J Appl Oral Sci. 2007 Jun;15(3):156-61. doi: 10.1590/s1678-77572007000300002. PMID: 19089123; PMCID: PMC4327460.
8. Babu NA, Mukherjee S, Rajesh E, Malthi L. DNA Profiling - Saviour in Forensic Odontology. European Journal of Molecular & Clinical Medicine.2020 Dec.14;7 (5):1447-55.
9. Lee SB, Shewale JG. DNA Extraction Methods in Forensic Analysis.2017: 1-15.
10. Sharma A, Sachdeva A, Bhateja S, Arora G. DNA Fingerprinting - An Asset to Forensic Dentistry. Biomedical Journal of Scientific & Technical Research.2018;9(5): 7375-78.
11. Jordan D and Mills D. Past, Present, and Future of DNA Typing for Analyzing Human and Non-Human Forensic Samples. Frontiers in Ecology and Evolution.2021;9: 646130.
12. Hartman D., Drummer O., Eckhoff C., Scheffer J.W., Stringer P. The contribution of DNA to the disaster victim identification (DVI) effort. Forensic Sci Int.2011;205(1-3): 52-58.
13. Sakari SL, Jimson S, Masthan KM, Jacobina J. Role of DNA profiling in forensic odontology. J Pharm Bioallied Sci. 2015 Apr;7(Suppl 1): S138-41. doi: 10.4103/0975-7406.155863. PMID: 26015692; PMCID: PMC4439652.
14. Chaudhary RB, Shylaja MD, Patel A, Patel A. DNA in forensic odontology: New phase in dental analysis. Int J Forensic Odontol.2020; 5(1):43-47.
15. Reynolds R, Sensabaugh G, Blake E. Analysis of genetic markers in forensic DNA samples using the polymerase chain reaction. Anal Chem. 1991 Jan 1;63(1):2-15. doi: 10.1021/ac00001a002. PMID: 1687345.

16. Bugawan TL, Saiki RK, Levenson CH, Watson RW, Erlich HA. The use of non-radioactive oligonucleotide probes to analyze enzymatically amplified DNA for prenatal diagnosis and forensic HLA typing. Biotechniques.1988; 6:943–47.
17. Linacre A, Templeton JEL. Forensic DNA profiling: state of the art. Research and Reports in Forensic Medical Science. 2014; 4:25-36. https://doi.org/10.2147/RRFMS.S60955.
18. Nims RW, Sykes G, Cottrill K, Ikonomi P, Elmore E. Short tandem repeat profiling: part of an overall strategy for reducing the frequency of cell misidentification. In Vitro Cell Dev Biol Anim. 2010 Dec;46(10):811-19. doi: 10.1007/s11626-010-9352-9. Epub 2010 Oct 7. PMID: 20927602; PMCID: PMC2995877.
19. Nwawuba Stanley U, Mohammed Khadija A, Bukola AT, Omusi Precious I, Ayevbuomwan Davidson E. Forensic DNA Profiling: Autosomal Short Tandem Repeat as a Prominent Marker in Crime Investigation. Malays J Med Sci. 2020 Jul;27(4):22-35. doi: 10.21315/mjms2020.27.4.3. Epub 2020 Aug 19. PMID: 32863743; PMCID: PMC7444828.
20. Azlina A., Zuraiah B., Ros S.M., Idah M.K., Rani. Extraction of mitochondrial DNA from tooth dentin: application of two techniques. Archives of Orofacial Sciences.2011; 9–14.
21. Nair MR, Shetty V, Suman P, Rao S, Sampath M, Subryahmanya RM. Gender Determination by Polymerase Chain Reaction Using Pulp Tissue of Deciduous Teeth Stored in Fresh Water and Natural Soil. World J Dent 2020;11(5):402–07.
22. Kasamatsu H, Robberson DL, Vinograd J. A novel closed-circular mitochondrial DNA with properties of a replicating intermediate. Proceedings of the National Academy of Sciences of the United States of America. 1971 Sep;68(9):2252-57. DOI: 10.1073/pnas.68.9.2252. PMID: 5289384; PMCID: PMC389395.
23. Syndercombe Court D. Mitochondrial DNA in forensic use. Emerg Top Life Sci. 2021 Sep 24;5(3):415-26. doi: 10.1042/ETLS20210204. PMID: 34374411; PMCID: PMC8457767.
24. Lim JJ, Khamis MF, Rashid NHA. Identification of Amelogenin Gene on Burnt Teeth Samples through Nested Polymerase Chain Reaction Amplification for Sex Identification. JSKM.2019;17(1): 91-98.
25. Chowdhury RM, Singhvi A, Bagul N, Bhatia S, Singh G, Goswami S. Sex determination by amplification of amelogenin gene from dental pulp tissue by polymerase Chain Reaction. Indian J Dent Res. 2018 Jul-Aug;29(4):470-76. doi: 10.4103/ijdr.IJDR_274_17. PMID: 30127199.
26. Kholief, M., El Shanawany, S. & Gomaa, R. Sex determination from dental pulp DNA among Egyptians. Egypt J Forensic Sci 7, 29 (2017). https://doi.org/10.1186/s41935-017-0030-x
27. Aal-Hamdan HMH, Refaat AM, Babu SR, Choudhry AR. Fetal gender determination through Y-STR analysis of maternal plasma during the third trimester of pregnancy. The Egyptian Journal of Medical Human Genetics.2015;16(1):47-53.
28. Khorate MM, Dhupar A, Ahmed J, Dinkar AD. Gender determination from pulpal tissue. J Forensic Dent Sci. 2014 May;6(2):107-12. doi: 10.4103/0975-1475.132540. PMID: 25125918; PMCID: PMC4130012.
29. Battepati, Prashant. Gender Determination using Primary teeth-A Polymerase Chain Reaction (PCR) study. Journal of dentistry and oral Hygiene.2013; 5(8): 77-82.
30. Mullis, K. B. and Faloona, F. A. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol.1987; 155:335–50.
31. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science. 1985 Dec 20;230(4732):1350-4. doi: 10.1126/science.2999980. PMID: 2999980.
32. Maulani C, Auerkari EI. Molecular analysis for sex determination in forensic dentistry: A Systematic Review. Egypt J Forensic Sci.2020;10(1): 1-9.
33. Girish K, Rahman FS, Tippu SR. Dental DNA fingerprinting in identification of human remains. J Forensic Dent Sci. 2010 Jul;2(2):63-68. doi: 10.4103/0975-1475.81284. PMID: 21731342; PMCID: PMC3125955.
34. Butler E, Li R. Genetic Markers for Sex Identification in Forensic DNA Analysis. J Forensic Investigation. 2014;2(3): 10.
35. Pakstis AJ, Speed WC, Fang R, Hyland FC, Furtado MR, Kidd JR, Kidd KK. SNPs for a universal individual identification panel. Hum Genet. 2010 Mar;127(3):315-24. doi: 10.1007/s00439-009-0771-1. PMID: 19937056.
36. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonák J, Lind K, Sindelka R, Sjöback R, Sjögreen B, Strömbom L, Ståhlberg A, Zoric N. The real-time polymerase chain reaction. Mol Aspects Med. 2006 Apr-Jun;27(2-3):95-125. doi: 10.1016/j.mam.2005.12.007. Epub 2006 Feb 3. PMID: 16460794.
37. Reesu GV, Augustine J, Urs AB. Forensic considerations when dealing with incinerated human dental remains. J Forensic Leg Med. 2015 Jan; 29:13-7. doi: 10.1016/j.jflm.2014.10.006. Epub 2014 Nov 13. PMID: 25572078.
38. Schwartz TR, Schwartz EA, Mieszerski L, McNally, Kobilinsky L. Characterization of deoxyribonucleic acid (DNA) obtained from teeth subjected to various environmental conditions. J Forensic Sci. 1991 Jul;36(4):979-90. PMID: 1680960.
39. Alkass K, Buchholz BA, Druid H, Spalding KL. Analysis of 14C and 13C in teeth provides precise birth dating and clues to geographical origin. Forensic Sci Int. 2011 Jun 15;209(1-3):34-41. doi: 10.1016/j.forsciint.2010.12.002. Epub 2011 Jan 3. PMID: 21208756; PMCID: PMC3105170.