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Forensic Science

TOOTH – A SOURCE OF DNA

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ABSTRACT Forensic science utilizes DNA analysis for human identification, with teeth providing a durable and unique DNA source. This review examines the role of forensic dentistry, highlighting extraction and analysis methods such as RFLP, STR typing, mtDNA, and Y chromosome analysis. Studies demonstrate dental DNA's resilience to environmental factors and the effectiveness of various extraction techniques. Despite challenges like DNA fragmentation and contamination, advancements in forensic odontology enhance identification accuracy, underscoring the importance of incorporating these technologies in forensic investigations.

KEYWORDS : DNA, Forensic Dentistry, DNA Analysis, STR Typing

INTRODUCTION

Human identification is a critical area of study and research within forensic science. It focuses on establishing the identity of individuals based on their physical characteristics. The groundbreaking discovery of the DNA double-helix structure by Watson and Crick in 1953 revolutionized scientific fields. This discovery paved the way for techniques that allow us to characterize an individual's uniqueness based on their DNA sequence. Fast forward three decades, and Jeffreys and colleagues (1985) introduced radioactive molecular probes capable of recognizing highly variable regions in DNA.[1] These unique patterns, known as DNA fingerprints, enable precise identification of each person.

Today, DNA profile tests are highly reliable and accepted as legal evidence in courts, including paternity cases and human identification. Various biological materials, such as bone tissue, hair bulbs, biopsy samples, saliva, and blood, can be used to isolate DNA for laboratory tests. While the quantity and quality of extracted DNA may vary across different tissues, it is possible to obtain DNA from virtually all parts of the human body.

Dna And Forensic Dentistry

Forensic Dentistry plays a crucial role in human identification processes. Under Brazilian Law no. 5081/66, dental professionals have the authority to investigate biological materials derived from the human body in various conditions (including quartered, dilacerated, carbonized, macerated, putrefied, skeletonized, and partially skeletonized remains) with the goal of establishing human identity.[2]

While fingerprints have historically been used for identification, they can be easily destroyed in situations like fire or skeletonization. In cases where dental records are available, experts often rely on them for identification. However, sometimes these records are incomplete or unavailable. Advances in biomolecular resources now allow us to identify individuals using small amounts of degraded biological material—a common occurrence in forensic analyses. For instance, after the South Asian tsunami disaster on December 26th, 2004, various techniques were employed to identify thousands of victims. These techniques included forensic pathology, forensic dentistry, DNA profiling, and fingerprinting. Remarkably, 99% of identifications were based on dental records or fingerprints, while only 1% relied on DNA profiling.[3,4]

Teeth play a critical role in identification and criminology due to their unique dental characteristics and their physical and chemical resilience. Even when conventional methods fail, teeth remain an excellent source of DNA, making them essential for identifying individuals.

Possible Applications Of Dna In Forensic Dentistry

The impact of environmental conditions on DNA extracted from dental pulps has been studied by Schwartz et al. (1991).[5] They explored variations in pH (ranging from 3.7 to 10.0), temperature (including tooth incineration), humidity levels (20%, 66%, and 98%), soil types (sand, potting soil, garden soil, submersion in water, and outdoor burial), and inhumation periods (from one week to six months). Remarkably, these environmental factors did not significantly affect the ability to obtain high-molecular-weight human DNA from dental pulp.

In another study, Malaver and Yunis (2003) evaluated different dental tissues as DNA sources in forensic analyses. They obtained 20 teeth from unidentified bodies buried in 1995 and exhumed in 2000, resulting in 45 DNA samples (5 from pulp, 20 from dentin, and 20 from cementum). The dental pulp yielded the strongest PCR amplification signals, while dentin and cementum signals were similar. Hanaoka et al. (1995) investigated DNA extraction from 50 teeth (pulpal and hard tissues). Dental pulp DNA ranged from 3 to 40 g, with no correlation between storage period and DNA amount. Hard dental tissues were efficiently analyzed only by PCR. Remualdo (2004) assessed PCR amplification of DNA from teeth subjected to heat (200°C, 400°C, 500°C, and 600°C) using different extraction methods. Organic extraction worked best at lower temperatures (200°C and 400°C), while isopropanol/ammonia acetate was effective at higher temperatures (500°C and 600°C), especially for mitochondrial DNA extraction.[6] An important case described by Sweet and Sweet (1995) involved human remains identification. Despite severe carbonization (reducing the body to 25% of its original

size), a preserved unerupted third molar allowed DNA extraction from the dental pulp (1.35 g), serving as an excellent source of high molecular weight genomic DNA.[7]

Beyond human identification, Forensic Dentistry also explores bite mark evidence. In cases of physical assault (e.g., sexual abuse, murders, and child abuse), bite marks are often found on the skin. Aggressors' saliva, deposited during biting, kissing, or suction, can be used for DNA analysis. While ABO blood group identification is possible in 90% of cases, DNA amplification techniques like STR profiling offer more informative results for identifying aggressors.

Guidelines For Obtaining Dna

To obtain dental tissue for DNA sampling, first, debride the tooth of any plaque or calculus using a curette, then wash thoroughly with hydrogen peroxide followed by ethanol.

For an intact tooth recently removed from the alveolus, perform conventional endodontic access and instrumentation. Open the access, and curette the walls of the pulp chamber with a slow rotary burr to collect the pulp tissue in a wide-open sterile tube. In dried specimens, the pulp may appear mummified and parchment-like. After instrumentation, irrigate the pulp chamber with buffer. Ultrafiltration of the liquid in the lab will remove the cellular material needed for analysis. Crushing the tooth may be necessary to locate cells containing useful DNA.[8]

Various techniques for retrieving DNA include crushing the entire tooth, performing conventional endodontic access, making a horizontal section with aggressive extirpation and apicectomy, and making a horizontal section with aggressive pulpectomy and crushing the radicular half of the tooth (Smith et al., 1993).[9] The "orthograde entrance" technique provides direct access from the enamel surface to the pulp cavity, allowing researchers to obtain more pulp and dentin-rich samples using endodontic files with coarser ridges.[10]

Researchers must carefully evaluate the conditions of the material to be examined, considering the risk of sample contamination and environmental influences. Typically, only small amounts of DNA are available, which may also contain PCR inhibitors. Environmental factors such as time, temperature, humidity (which can promote microorganism growth), light (both sunlight and UV light), and exposure to various chemicals can lead to DNA degradation.[11]

METHODS OF DNA ANALYSIS

A. Restriction Fragment Length Polymorphism (rflp)

RFLP is used to analyze the variable lengths of DNA fragments that result from digesting a DNA sample with an enzyme called "restriction endonuclease," which cuts DNA at specific sequence patterns known as restriction endonuclease recognition sites.[11]

RFLP cannot be performed on samples degraded by environmental factors and takes a longer time to produce results because it requires relatively large amounts of DNA. If a mutation occurs within the restriction enzyme's target site, the enzyme cannot recognize and cut the DNA, leaving the two pieces together. This uncut, longer fragment migrates more slowly through the gel compared to the two shorter fragments.

Variations in the distance between two restriction enzyme sites can occur through insertions, deletions, or changes in the number of repeating units, which is particularly useful in forensic science.[8] In a study by Y. J. Zhang et al., multiple RFLP genotypes of Porphyromonas gingivalis were found to colonize a single periodontal pocket.[11]

Forensic application of PCR-RFLP genotypic comparison of Streptococcus mutans has been used to recover DNA from bite injuries, playing an important role in forensic odontological analysis.[12]

B. Short Tandem Repeats (STRs)

Typing Short Tandem Repeats (STRs) are short stretches of DNA repeated at various locations throughout the human genome, used to assess specific regions (loci) within nuclear DNA.[11]

STRs have a high power of individual identification due to their high polymorphic informative content. The non-overlapping size of alleles from different contributors helps differentiate them. Modern detection methods involve fluorescent detection using capillary or gel electrophoresis and ABI gel-based DNA sequencers, while earlier methods used silver-stained polyacrylamide gels.

STRs are employed in paternity testing because each individual inherits some STRs from their father and some from their mother. These hyper-variable regions show repetitions of fragments with 2–7 base pairs and aid in identifying victims of mass disasters from old remains.[13]

In a study by Shbair M et al., the reliability of STR markers in the carious part of teeth was evaluated in 120 carious teeth, revealing that carious tissues of human teeth are as valid as healthy teeth for forensic human identification.[14]

C. Mitochondrial DNA (mtDNA) Analysis

In a study by Ginther C et al., mtDNA was extracted from teeth stored for periods ranging from 3 months to 20 years.

Tooth donors or their maternal relatives provided blood or buccal cells from which mtDNA was also extracted. Enzymatic amplification and direct sequencing of approximately 650 nucleotides from two highly polymorphic regions of mtDNA produced identical sequences for each comparison between tooth and fresh DNA. This suggests that teeth are an excellent source of high molecular weight mtDNA, which can be valuable for extending the time frame during which decomposed human remains can be genetically identified.[15]

D. Y Chromosome Analysis

The majority of the length of the human Y chromosome is inherited as a single block in linkage from father to male offspring, functioning as a haploid entity. Thus, Y chromosomal DNA variation is mainly used in investigations of human evolution, forensic purposes, and paternity analysis.

In a study by Tsuchimochi T et al. (2002)[16], DNA was extracted from dental pulp using the chelex method, amplified with PCR, and typed at Y-chromosomal loci to determine the effects of temperature on the sex determination of teeth.[17]

Whittaker and colleagues used quinacrine mustard staining and fluorescent Y chromosome testing to determine sex from necrotic pulp tissue, claiming that sex determination can be accurately completed up to five weeks after death. Duffy et al. demonstrated that Barr bodies and Y chromosomes (F bodies) are preserved in dehydrated pulp tissues for up to one year, and that pulp tissues retain sex diagnostic characteristics even when heated to 100°C for one hour.[18]

E. X-Chromosome STR Analysis

Analysis of ChrX short tandem repeat markers (STRs) can effectively address challenges encountered in certain cases of relationship analysis, particularly when the offspring is female. Fathers transmit the same X chromosome to all their daughters, making ChrX STRs particularly useful in deficiency paternity cases involving female children, maternity testing, and paternity cases involving blood relatives.[12]

ChrX STRs serve as a complementary tool to autosomal STR and mitochondrial DNA (mtDNA) markers, with higher mean exclusion chance (MEC) values observed in trios involving daughters.[19] Due to their small allele size (generally 100-350 nucleotides), X chromosome STRs are easily amplified and detected with high sensitivity, making them a powerful complementary system, especially in deficiency paternity testing.[11]

Hanaoka et al. (1996)[20] 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. This method proved to be useful in determining the sex of an individual.

Limitations Of Tooth Dna Fingerprinting

DNA typically undergoes fragmentation after death due to enzymatic activity, such as that of DNAases. Obtaining DNA from remains is challenging due to various factors, including the condition of the remains, the time elapsed since death, storage and transportation conditions, and contamination. Another difficulty lies in obtaining usable DNA from all available DNA in the remains.

Before DNA extraction, teeth commonly undergo decontamination using sodium hypochlorite to remove soft-tissue remnants, which can affect the DNA content. There is a high risk of contamination during collection, storage, and transportation of samples. The type and condition of available teeth, as well as the tooth structure used, can also impact DNA quality and hinder DNA fingerprinting.[21]

CONCLUSION

The 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.

REFERENCES

1. Silva RHA da, Sales-Peres A, Oliveira RN de, Oliveira FT de, Sales-Peres SH de C. Use of DNA technology in forensic dentistry. Journal of Applied Oral Science [Internet]. 2007 Jun;15(3):156–61.
2. Oliveira RN, Daruge E, Galvão LCC, Tumang AJ. Contribuição da odontologia legal para a identificação "post-mortem". Rev Bras Odontol. 1998;55(2):117–122
3. Lau G, Tan WF, Tan PH. After the Indian Ocean Tsunami: Singapore's contribution to the international disaster victim identification effort in Thailand. Ann Acad Med Singapore. 2005;34(5):341–351
4. Morgan OW, Sribanditmongkol P, Perera C, Sulasmi Y, Van Alphen D, Sondorp E. Mass fatality management following the South Asian Tsunami Disaster: case studies in Thailand, Indonesia, and Sri Lanka. PLoS Med. 2006;3(6):809–815
5. Schwartz TR, Schwartz EZ, Mieszerski L, McNally L, Kobilinsky L. Characterization of deoxyribonucleic acid (DNA) obtained from teeth subjected to various environmental conditions. J Forensic Sci. 1991;36(4):979–990
6. Malaver PC, Yunis JJ. Different dental tissues as source of DNA for human identification in forensic cases. Forensic Sci. 2003;44(3):306–309
7. Sweet D, Sweet CHW. DNA analysis of dental pulp to link incinerated remains of homicide victim to crime scene. J Forensic Sci. 1995;40:310–314
8. 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.
9. 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
10. 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.
11. 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.
12. Zhang YJ, Yasui S, Yoshimura E, Ishikawa I. Multiple restriction fragment length polymorphism genotypes o/Porphyromonas gingivalis in single periodontal pockets. Oral Microbiol Immunol. 1995;10(2):125–8.
13. 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.
14. 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.
15. Ginther C, Tarver LI, King M. identifying individuals by sequencing mtDNA from teeth. Nat Genet. 1992;2(2):135–8.
16. Tsuchimochi T, Koyama H, Matoba R. Chelating resin-based extraction of DNA from dental pulp and sex determination from incinerated teeth with Y-chromosomal alphoid repeat and short tandem repeats. Am J Forensic Med Pathol . 2002;23(3):268–71.
17. 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
18. Whittaker DK, Lewelyn DR, Jones RW. Sex determination from necrotic pulpal tissue. Bri. Dent J. 1975;139(10):403–5.
19. 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.
20. Hanaoka Y, Minaguchi K. Sex determination from blood and teeth by PCR amplification of the alphoid satellite family. J Foren Sci. 1996;41(5):855–63.
21. Afziya Waseem Qadri, Yadav S, Jain A, Devi Charan Shetty, Gulati N. Tooth as a vital source of DNA in forensic odontology: Recent perspective. Journal of Academy of Dental Education. 2023 Dec 9;9:73–9.