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Human identification using dental DNA in crime scene investigations: from genetic markers analysis to forensic extraction

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ABSTRACT

The objective of this study was to review the updated literature on the importance of DNA in human identification and on the possibility of extracting DNA from teeth in forensic contexts. The review highlights scientific progress from the development of microscopy to research on the human genome, including the process automation, the analysis of genetic markers, and DNA extraction from teeth. In addition, recent technological advances are discussed and current methods used for DNA-based human identification at crime scenes are described, including Rapid DNA technology, forensic genetic genealogy, forensic DNA phenotyping, and DNA extraction from teeth. In conclusion, DNA-based human identification is fundamental to forensic practice because it supports case resolution and the establishment of kinship relationships. Therefore, staying updated in this field is essential to effectively apply scientific advances to clinical investigations.

Keywords: forensic genetics; human identification; genetic profile; tooth; forensic odontology.

INTRODUCTION

Human identification using DNA in crime scene investigations represents a significant advancement in forensic investigation, as it provides legal and forensic professionals with an accurate tool for individual identification and the elucidation of criminal acts. The genetic information contained in human DNA is unique to everyone; therefore, it constitutes a marker that allows people to be distinguished with a high degree of precision. Bloodstains found at scenes of violent crimes constitute relevant forensic evidence, as they enable the reconstruction of events. However, in certain cases, experts must contend with a scarcity of samples and rely on presumptive tests before DNA analysis, with the risk that such samples may be discarded.

Given the increasing incidence of crimes against persons, it is important to optimize the storage of samples to prevent DNA degradation (1-3). This identification method has become a cornerstone in crime resolution, as it offers an unprecedented capacity to establish definitive links between a suspect, a victim, and the crime scene. Since its introduction, DNA identification tech-

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nology has transformed the way criminal investigations are approached, providing irrefutable evidence that can be crucial in determining an individual's guilt or innocence (4, 5).

The objective of this study is to conduct an updated review of the literature on the importance of DNA in human identification and its potential recovery from dental tissues in forensic contexts.

LITERATURE REVIEW

A comprehensive search was conducted in the PubMed, Scopus, EBSCO, and Google Scholar databases using Medical Subject Headings (MeSH) and Health Sciences Descriptors (DeCS) as search terms, including: "forensic genetics," "human identification," "genetic profile," "tooth," and "forensic dentistry." Original articles, reviews, systematic reviews, and meta-analyses published between 2018 and 2023 were included. A total of 20 relevant articles were selected for the review, allowing the integration of updated, high-quality scientific evidence from multiple sources and with different methodological approaches.

Development of human identification by DNA at the crime scene

Human interest in exploring living organisms has driven the study of genetics from ancient philosophers to Mendel and his laws of inheritance. The microscope enabled detailed observation of cells and chromosomes. During the 20th and 21st centuries, attempts were made to replicate species—such as the famous cloning of Dolly the sheep—and to map the human genome, to improve health and quality of life (6).

DNA, or deoxyribonucleic acid, is the fundamental genetic material of the human genome and is essential for the creation and maintenance of organisms. Its double-stranded nucleotide structure forms a double helix. The bases adenine (A), cytosine (C), guanine (G), and thymine (T) transmit genetic information. DNA is crucial for human identification and for the diagnosis of diseases through genetic and epigenetic alterations (6). In this regard, DNA testing, which has greater discriminatory power than fingerprints or traditional markers, is essential for identifying and solving crimes, as it provides certainty to incriminating evidence (7).

In forensic science, the development of methodologies and genetic markers has advanced significantly. From the introduction of classical markers, such as blood groups, to the incorporation of more sophisticated techniques, such as Alec Jeffreys' genetic fingerprinting in 1985, there has been an evolution towards methods based on DNA analysis, such as Restriction Fragment Length Polymorphism (RFLP), Multilocus Probes (MLP), and Variable Number of Tandem Repeats (VNTR).

RFLP analysis detects variations in DNA sequences using restriction enzymes, while MLP enables the identification of multiple polymorphic loci in a single assay. Despite their initial usefulness, VNTR—DNA sequences in which a short nucleotide segment is repeated a variable number of times—were replaced by Single-Locus Probes (SLP), which detect variation at a single locus and overcome limitations related to interpretation and reproducibility. On the other hand, the polymerase chain reaction (PCR), developed by Kary Mullis in 1983, allows efficient DNA amplification even from minimal samples, such as single cells, facilitating forensic identification using VNTR markers and later short tandem *repeats* (STR). Consequently, short repetitive sequences have revolutionized forensic genetics by enabling efficient amplification from minimal amounts of DNA and facilitating automation and rapid generation of genetic profiles from simple biological samples such as blood or saliva (8).

Use of rapid DNA technology at the crime scene

In contemporary forensic investigation, Rapid DNA technology has emerged as a revolutionary tool. Designed to automate DNA profile generation from buccal samples, this technology simplifies the analytical process and significantly reduces reliance on specialized personnel. The Accelerated Nuclear DNA Equipment (ANDE), approved by the FBI's National DNA Index System (NDIS) in 2018, integrates the ANDE 6C instrument, A- and I-chips, and Expert System software. It also employs the multiplex FlexPlex27 assay, allowing the simultaneous evaluation of 27 STR loci. The resulting DNA profiles can be automatically searched against the FBI database through the Rapid DNA Index System (RDIS).

ANDE has demonstrated its effectiveness in the rapid identification of forensic samples, including human remains and small biological samples, as evidenced by its performance during the 2018 Camp Fire in California. This system was fundamental for the rapid identification of victims in record time after their recovery. In addition, in the USA and globally, law enforcement agencies and the military have implemented it to combat crimes such as human trafficking and sexual assault (1).

Despite its documented effectiveness, the implementation of Rapid DNA technology faces significant challenges, including variability in sample type and quality, environmental exposure, and DNA age. These limitations must be addressed to maximize the technology's effectiveness and reliability in critical forensic applications (2).

Forensic genetic genealogy

Forensic Genetic Genealogy (FGG), developed since 2018, combines family tree reconstruction with genetic analysis. This innovative methodology has been successfully applied in numerous unsolved cases in the United States, providing clues and enabling the identification of unknown individuals. The proliferation of DNA testing services and public interest in genetic ancestry have expanded access to human genetic data. Currently, public genetic genealogy databases exist worldwide. Genetic genealogy, used by amateurs and professionals, analyzes genetic connections within family trees using consumer DNA data (9).

Michael et al. (10) highlight the collaboration among anthropologists, forensic genealogists, and police officers in identifying a homicide victim from a case that had remained unsolved for over a century. Remains discovered in 1979 and 1991 were identified in 2019 through genetic genealogy, analyzing bone microstructure, trauma, and historical evidence. This case highlights the importance of integrating advanced forensic techniques to solve historical crimes. Thus, FGG combines advanced DNA sequencing with genealogical methods to resolve violent crimes and identify human remains, thereby expanding forensic evidence approaches.

Forensic DNA phenotyping

Phenotype refers to the physical manifestation of the genotype. Forensic DNA phenotyping (FDP) involves genetic analysis that predicts physical characteristics of unknown or deceased individuals based on biological samples obtained at the crime scene. This approach complements traditional DNA analysis based on STR markers, which is essential but limited when there are no matches in the databases.

By using single nucleotide polymorphisms (SNP), FDP overcomes the limitations of STR multiplexing and allows traits such as ancestry, height, skin, eye, and hair color to be inferred. Thus, SNPs, which are abundant and amenable to automation, enhance forensic analytical precision and the identification of unknown individuals (3).

Forensic DNA analysis compares genetic profiles obtained at crime scenes with those of suspects stored in databases, facilitating the identification of unknown offenders and the linkage of crimes. However, when no STR matches are identified, additional genetic testing becomes crucial for case resolution and suspect apprehension (5). In this regard, SNP analysis is promising for studying phenotypic variability and biogeographic origin, reducing the number of potential donors to a smaller group with visible characteristics and inferred DNA ancestry.

DNA extraction from teeth

DNA-based identification of human remains plays an important role in forensic science; however, environmental factors can affect molecular preservation. In this context, the involvement of forensic dentists in crime scene investigations is essential. Teeth, due to their resistance to adverse environmental conditions, have become fundamental in postmortem analysis, enabling age estimation, blood group determination, and identification in mass disaster scenarios. In addition, they facilitate the correlation between bite marks and the suspect's dental impressions. Accordingly, considerable attention has been given to methodologies for extracting DNA from hard tissues because of their longevity.

Forensic DNA profiling through STR typing enables genetic discrimination among individuals. This is largely based on comparative analyses with profiles of known individuals, which may be seen as a limitation in some cases. Nevertheless, it remains the most reliable identification method, particularly when used in conjunction with dentistry and fingerprinting (7,11).

Although DNA is an optimal tool for identifying human remains, certain postmortem environments are unfavorable for its preservation. As decomposition progresses, the availability of high-yield DNA sources decreases; therefore, bone and tooth samples are often preferred due to their durability across a wide range of postmortem conditions. Hard tissue samples (bones and teeth) undergo much more laborious processes to extract the DNA molecule; therefore, research to improve and facilitate the successful extraction of DNA from this type of sample is important, considering that many humanitarian and medico-legal cases involve human skeletal remains.

Bone is one of the strongest materials, and enamel—the outer layer of the tooth crown—is the hardest tissue in the human body, with resistance properties comparable to steel. However, due to its largely avascular nature, enamel is not considered a useful source of DNA. In contrast, other regions such as the vascularized pulp, cementum, and dentin contain odontoblasts, cementocytes, and fibroblasts, making them valuable DNA sources (12). Gawali et al. (13) evaluated the suitability of dental tissues and alveolar bone as DNA sources for forensic investigation, revealing that dentin is the most reliable DNA source, exhibiting low levels of heterozygote imbalance; dental pulp provides the highest DNA yield, albeit with significant heterozygote imbalance; cementum is less favorable; and alveolar bone shows high DNA yield and serves as a reliable alternative source for forensic analysis.

Regarding tooth sampling for DNA extraction, various procedures have been described, including horizontal sectioning of teeth at the cementoenamel junction, vertical sectioning extending to the root apex, as well as scraping and aspiration techniques. In the modified horizontal section method, the tooth is marked circumferentially 1 mm below the cementoenamel junction with a long-handled round bur, leaving a 2- to 3-mm-wide isthmus of intact tooth structure on the buccal surface. This method offers several benefits, including simplicity, accessibility, preservation of both the crown and root of the tooth, and the possibility of restoring the tooth to near-original condition (14).

Another method involves horizontally sectioning the tooth, removing the dental pulp from the chamber, and grinding the remaining root into a fine powder, which can be used separately for DNA extraction. On the other hand, tooth grinding or cryogenic grinding allows sufficient genetic material recovery using a freezer mill that pulverizes teeth under sterile and cryogenic conditions with liquid nitrogen; however, this procedure may result in the complete destruction of the dental sample (14).

Given this scenario, in recent years, conservative methods that preserve dental samples rather than sectioning the tooth have gained importance, particularly for archaeological or museum specimens. Dental pulp, which is rich in genetic material, can be collected by accessing the pulp chamber through conventional endodontic access cavity preparation. This technique is notable for its simplicity, low cost, and preservation of dental integrity, making it especially relevant in forensic investigations (14).

In forensic studies, dental remains are critical when adequate soft tissue is unavailable. In this context, Carrasco et al. (15) evaluated the effectiveness of a workflow for nuclear DNA analysis from dental samples in cases involving missing persons or disaster victims where soft tissue was absent. Traditionally, these samples yield low-quality or low-quantity DNA; therefore, the proposed workflow utilizes the DFK^{MR} extraction kit and the QuickExtract™ FFPE kit to enhance DNA recovery, along with the InnoQuant® HY and ForenSeq® kits for sensitive quantification and simultaneous genotyping of 231 STR and SNP markers. Preliminary results using degraded blood samples were promising, demonstrating the system's sensitivity. The method showed success with dental samples having postmortem intervals ranging from 8 days to 6 months, providing informative SNP and STR DNA profiles and highlighting its utility under adverse conditions for forensic identification.

In the study by Heathfield et al. (16), 52 teeth from three deceased individuals were analyzed using the QIAamp® DNA Investigator kit and phenol–chloroform extraction. Contrary to conventional recommendations, premolars and canines yielded better DNA profiles than molars. The presence of dental restorations negatively affected DNA quantity and quality, potentially explaining the poorer performance of molars. Teeth affected by periodontitis exhibited significantly improved DNA concentration, integrity, and profiling success compared to healthy teeth, possibly due to increased leukocyte presence at the site. Similarly, no significant differences in DNA profiling success were observed between the two extraction methods. The acquisition of additional DNA profile information from degraded samples may contribute to reducing the burden of unidentified human remains.

Overall, the application of DNA technology has revolutionized forensic identification procedures since its emergence more than 35 years ago. Based on the evidence reviewed, it can be concluded that teeth—owing to the high quality of DNA they preserve compared to other body tissues—constitute a fundamental resource in forensic investigation, standing out as a valuable genetic source that continues to drive and expand research in this field.

DISCUSSION

In 1983, Kary Mullis developed the polymerase chain reaction (PCR) to amplify DNA, revolutionizing molecular biology and forensic identification. Subsequently, short tandem repeats (STR), which are readily amplifiable, enhanced sensitivity and robustness with minimal quantities of DNA (8). Currently, rapid DNA analysis has been developed for direct application at crime scenes. Rapid DNA technology, such as the ANDE system, automates the generation of DNA profiles from buccal swabs, eliminating the need for extensive analyses (1). In this context, the Rapid DNA Act of 2017 in the United States stimulated interest in rapid DNA technology for forensic analysis (2).

The study by Bowman et al. (17) supports the forensic application of Rapid DNA, highlighting its effectiveness in disaster victim identification. Moreover, this technology enables DNA profiling at the time of arrest, reducing the need for analyst review. Its use has been explored both without review and in modified workflows, and it has been successfully applied at crime scenes and to postmortem samples for the identification of deceased individuals, as demonstrated during the 2018 California wildfire.

Since 2018, forensic genetic genealogy (FGG) has played a pivotal role in criminal investigations in the United States, as it leverages genetic data from consumer DNA testing to solve violent crimes and to establish the identity of previously unidentified human remains, as in the case of a man murdered in 1916 (9,10). The Golden State Killer case (2018) highlighted the search for genetic profiles in GEDMatch, a genealogical database. The police, in collaboration with a genealogist, used this approach to identify Joseph James DeAngelo through discarded DNA samples (18). This case highlights both the relevance and the potential ethical impact of genetic databases in criminal investigations.

Regarding genotyping for forensic DNA phenotyping (FDP), in 2019, Meng et al. (19) developed a method capable of identifying externally visible characteristics (EVC) when other approaches fail. They employed a "molecular witness" to infer traits such as hair and eye color from biological samples. This aligns with the research study conducted by Dabas et al. (3). However, according to Ragazzo et al. (5), forensic DNA analysis involves comparing genetic profiles obtained at crime scenes with those of suspects or genetic databases. In the absence of suspects, these databases facilitate the identification of unknown offenders and the linkage of crimes, particularly in cases involving serial offenders. When no STR profile matches are obtained, police investigations may rely on additional genetic tests. On the other hand, advances in FDP have improved the understanding and prediction of traits such as height, skin, eye, and hair color, facial features, and associated genetic variations. In this regard, ongoing research in molecular genetics promises rapid developments in molecular DNA-based identification for the characterization of human phenotypes (3,19).

DNA extraction methods from teeth are critical in postmortem analyses due to their resistance. Focusing on hard tissues such as bones and teeth, STR typing provides high accuracy but remains dependent on known comparisons. DNA extraction from hard tissues—particularly dental pulp and dentin—is essential in humanitarian and medico-legal cases (7,11,12). Carrasco et al. (15) overcame difficulties in the extraction of dental DNA with the use of the DFK^{MR}, QuickExtract™ FFPE, InnoQuant® HY, and ForenSeq® kits, achieving successful genotyping. Similarly, Heathfield et al. (16) demonstrated that canines and premolars yielded the best DNA profiles.

Age estimation in forensic dentistry is vital to prevent injustices, particularly in immigrants. In this regard, Tejasvi et al. (20) propose measuring telomere length (TL) using quantitative real-time PCR. This pioneering study estimates age using telomeric DNA from dental pulp, representing an innovative method that enhances the accuracy of legal procedures.

Among the limitations identified, although technological advances have been fundamental to human identification by DNA at crime scenes, most studies have been conducted in developed countries with technological, economic, and regulatory realities that differ from those of our context, limiting the generalizability of results. Therefore, there is a need to implement policies and regulations governing these technologies to advance in parallel with the global context, for the benefit of the population and in the fight against crime.

CONCLUSIONS

Human identification by DNA at the crime scene has undergone remarkable development and advancement in recent years. Accordingly, methods such as forensic genetic genealogy (FGG) have emerged, integrating advanced DNA sequencing technologies with genealogical approaches. Forensic DNA phenotyping (FDP), by employing single nucleotide polymorphisms (SNP) rather than short tandem repeats (STR), enhances identification in cases with no STR matches, optimizing investigations and narrowing the pool of potential donors when no suspects have been identified. Rapid DNA analysis at the crime scene has also demonstrated significant progress; however, it still faces challenges related to DNA variability and quality, which must be addressed to ensure forensic effectiveness and accuracy. Finally, one of the most promising methods is DNA extraction from teeth. Despite the challenges associated with this approach, the application of forensic kits and improved techniques—such as the use of QuickExtract™ and massively parallel sequencing—has shown substantial advances in the recovery and genotyping of dental samples, thus contributing to identification in forensic cases.

Teeth provide an excellent source of high-quality DNA compared to other body tissues, which is why they are consistently considered in forensic investigations. In this regard, this review highlights the significance and the need for further research in forensic science and forensic dentistry, with the aim of strengthening national and global security. This is particularly relevant given that many countries possess extensive DNA databases that facilitate comparison and human identification. Therefore, in the Latin American context—and specifically in Peru—it is essential to implement these technologies and to promote further scientific research in this field to contribute to the fight against crime and to the pursuit of justice.

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