International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

A Comprehensive Review of DNA Extraction and Analysis from Buried Human Remains

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ABSTRACT
Deoxyribonucleic Acid (DNA) stands as a cornerstone of modern forensic science, offering unparalleled precision in human identification and kinship analysis. DNA's unique molecular signature, inherited from both parents, has revolutionized criminal investigations, disaster victim identification, and paternity testing. The process of DNA extraction, quantification, and analysis, wherein genetic material is isolated from biological samples and subsequently amplified and examined through techniques such as Polymerase Chain Reaction (PCR), Short Tandem Repeat (STR) analysis, and next- generation sequencing (NGS). The admissibility of DNA evidence from exhumed bodies in Indian courts is governed by Indian Evidence Act, 1872, with judicial precedents affirming its scientific credibility. In scenarios of unidentified remains or historical investigations, exhumation becomes necessary. Exhumation, the legal process of disinterring human remains, plays a critical role in facilitating DNA-based identification. In India, exhumation is governed primarily by the Code of Criminal Procedure (CrPC), 1973, which mandates magistrate oversight, ensuring legality, procedural propriety, and respect for cultural sensitivities. The process is inherently complex due to factors such as advanced decomposition, burial environment, and time since interment. These factors significantly affect the quality and viability of DNA that can be extracted from bone, teeth, and other tissues. Several challenges in the exhumation and DNA analysis process in India are manifold. Environmental conditions such as high humidity, temperature extremes, and microbial activity contribute to DNA degradation. Inadequate infrastructure, delays in authorization, and lack of standardized protocols across jurisdictions further hinder timely and effective processing. Ethical and religious concerns, particularly in communities where disturbing the dead is taboo, can also impede exhumation efforts. Forensic teams often encounter logistical constraints like insufficient protective equipment and poor coordination among law enforcement, judiciary, and forensic labs. In conclusion, while exhumation and DNA analysis from decomposed remains present considerable scientific and logistical challenges in the Indian context, advancements in forensic methodologies and judicial openness to genetic evidence have made significant strides. Continued investment in infrastructure, training, and legal reform is imperative to harness the full potential of DNA technology in serving justice and identifying the unknown. The integration of scientific rigor with ethical and cultural considerations remains vital in the evolving landscape of forensic investigation in India.

Introduction

History of DNA

Fredrich Miescher first attempted to separate the cells from lymph nodes for his research, but it was challenging to purify the lymphocytes and impossible to get enough. Since proteins were thought to be the most promising candidates for comprehending how cells function, Miescher initially concentrated on the many kinds of proteins that comprise leucocytes. Miescher sought to categorize them, provided a detailed description of their characteristics, and demonstrated that proteins and lipids made up the majority of the cells' cytoplasm. The variety of proteins found in the cells outshone his analytical techniques, and his study was limited by the basic procedures and tools at his disposal. However, Miescher observed during these experiments that a material dissolved once again when alkali was added and precipitated out of the solution when acid was introduced. For the first time, he had managed to obtain a crude DNA precipitate. He chose to look more closely at the cells' nuclei, a section of the cell about which not much was known at the time, because Miescher said that he had to attribute such material to the nucleic in accordance with known histochemical facts. Other researchers began studying nuclein after Miescher's original description in his 1871 publication. [1]

Figure 01: Glass vial containing nuclein isolated from salmon sperm by Friedrich Miescher while working at the University of Basel. The faded label reads "*bNuclein aus Lachssperma"*, F. Miescher (Nuclein from salmon sperm, F. Miescher). [2]

Most chemists were interested in Miescher or Hoppe-Seyler, frequently through personal interactions. The most famous discovery made by Albrecht Kossel, a fellow researcher at Hoppe-Seyler's lab and eventual Nobel Prize winner in medicine, was that nuclein was made up of four bases and sugar molecules. Nuclein continued to receive relatively little attention for a long time after Miescher's passing. Genetic information must be carried by proteins. While DNA is made up of only four different nucleotides, too few, it was thought, to store the vast amount of genetic information, proteins are made up of 20 different amino acids. James Watson and Francis Crick (1953) gave the first explanation of how DNA functions when they were able to decipher its structure, proposing a double helical structure of DNA. The greatest breakthroughs in field of forensic science are the invention of DNA sampling, profiling and analysis. Each and every DNA is unique and leaves indelible traces of ourselves, greatly assisting the police officer and forensic scientists to identify victims and perpetrators as well.[2]

Structure of DNA

In 1889, Altmann demonstrated that the nuclein could be divided into two pieces, which helped to clarify its nature. materials: a phosphorus-rich organic acid known as nuclein acid, which is later referred to as nucleic acid; and other proteins, which are classified as a type of albumin. There are two different types of nitrogenous base pair named as purine and pyridine, which includes Adenine(A) and Guanine(G), Thymine(T) and Cytosine(C) respectively. These nitrogenous bases are bonded with a double and triple hydrogen bond. The double- helical structure designed as an anti- parallel direction includes 3'- 5' direction and 5'- 3'direction. [3]

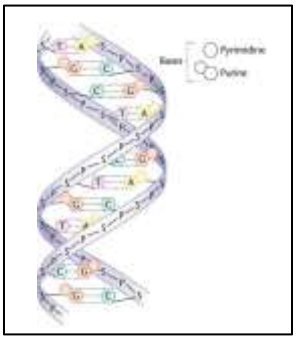

Figure 02: Double- Helical structure of DNA, paired with the two different groups as purine and pyrimidine with double and triple hydrogen bonds respectively.[3]

DNA Evidence

DNA evidence must always be accurately and properly collected, preserved, and documented in order for the court to be convinced that the evidence presented is trustworthy. Only then may it be admitted into evidence. No particular law exists in India that can give precise instructions to the court and investigating authorities regarding the process to be followed in cases involving DNA as evidence. [4]

The source of the DNA taken from the body changes according to the amount of time that has elapsed after the point of expiration when using short tandem repeat (STR) analysis for personal identification. White blood cells are used to extract DNA if less than twenty- four hours have elapsed. DNA is usually extracted from cartilage for victims discovered between 2–5 days of death; if more than 5 days have elapsed, bone and other hard tissues are the last resort. [5]

DNA analysis is used to identify the individuals based on their unique genetic makeup. It plays a crucial role in solving the different types of Criminal wrongful activities. Samples of DNA is being collected and extracted from the bodily fluids such as blood, semen, saliva, and other samples which includes skin cells, hair, bone, etc., which helps to identify or linking out the suspects or victims through the crime scene for further investigation process. These samples are also being used to establish out the paternity and maternity relationships. A large number of victims are introduced in the natural disasters such as flood, earthquake, etc. and even in wars and terrorist attacks, through the analysis of DNA samples it is easy to identify the individuals and matches DNA from unidentified remains with the family members. Moreover, DNA evidence helps to exonerate the innocents which can overturn wrongful convictions.

Figure 03: The right femur of unidentified human skeletal remains recovered in 2012 was utilized to get a DNA sample for the identification of the subject.[6]

DNA Extraction

Organic method (Phenol- Chloroform)

In organic method the phenol- chloroform extraction of DNA is the first method for the extraction of DNA. This method involves the liquid-to-liquid extraction, means using of two different solutions to separate

with respect to their different solubilities. The procedure initiates from the breaking down the cells for exposing out the DNA using a solution of SDS (sodium dodecyl sulphate) and proteinase K. in order to remove proteins from solution, phenol- chloroform is equally added to the solution.
Centrifugation is followed up to separate out two different layers, the lower phase and upper aqueous phase which consisting of required DNA sample. the ethanol and isopropanol are used for precipitation to remove the inhibitors like hemoglobin (Hb). While working in the organic extraction method it requires high molecular weight DNA for well-functioning, it consumes more time and involves the use of hazardous chemicals and requires the sample transferring into several tubes which increases the risk of error or contamination. [7]

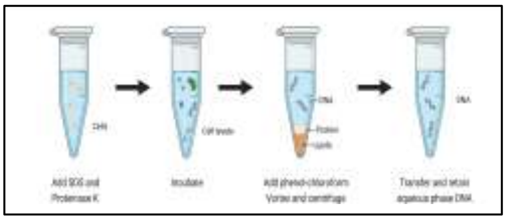

Figure 04: The PCIA method for extracting organic DNA is shown in this illustration. PCIA separates DNA into the aqueous phase, whilst lipids and proteins are partitioned into the organic and interphases, respectively.[8]

Chelex Method
Since extraction only needs a tiny bit of DNA, it is far more useful to forensic sciences. This technique involves adding a chelating resin dispersion, known as Chelex, straight to the DNA sample that was obtained. Chelex deactivates nucleases, which are enzymes that break down DNA, by binding metal ions. Consequently, the male's DNA molecules are shielded, allowing us to remove them from the specimen. The extraction process involves boiling the cell samples with a 5% Chelex solution to rupture the cells and liberate the DNA. The majority of the debris that can potentially obstruct analysis is bound by the Chelex resin. It can bind the proteins inside the blood sample, the heme, magnesium and metal ions. After that, the sample is heated to 100 degrees Celsius, which is high enough to denature DNA. Enzymes and results in the single-stranding of the double-strand structure.
This material can be centrifuged because the high temperature also breaks down the proteins that endanger cell membranes. The liquid layer containing DNA is then eliminated when the solution is rapidly spun. When the ions and debris bond to the beads, it indicates that the DNA is free to be extracted from the solution. Compared to the phenol chloroform extraction procedure, Chelex extraction is simpler, less expensive, and safer to use. Compared to previous approaches, the method's risks of contamination are significantly lower because it may be used in a single troop. But there is a drawback to Chelex in terms of examining the DNA. Since Chelex denatures DNA and turns it into a single strand, it is ineffective at limiting the length of DNA fragments. Although single- stranded DNA can be used with PCR, the issue with Chelex extraction is that not all of the PCR analysis's inhibitors are eliminated when using the Chelex procedure.[9]

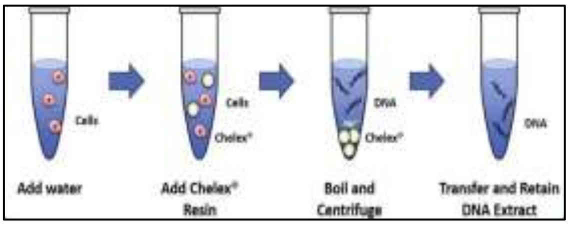

Figure 05: Procedure of DNA Extraction from Chelex DNA extraction method.[10]

FTA Card Method

FTA paper is cellulose-based absorbent paper that has chemicals that shield DNA molecules from being broken down by nucleases and stop development of bacteria DNA on FTA paper is hence stable. Using FTA® Purification Reagent to wash the cards (Whatman) in accordance with the manufacturer's instructions, DNA was recovered from blood stains collected on FTA® cards (Whatman, Kent, UK). After being spotted onto an FTA® card, a drop of 150 µl of fresh whole blood was allowed to dry at room temperature. A card with dried blood inside a circle 1.2 mm in diameter was over a number of years at room temperature. Consistent results can be obtained without quantification (used directly), which is a major advantage of FTA papers, without needing to measure the optimal quantity of DNA). extracted using a Harris micro punch from the FTA® card and put in an Eppendorf PCR tube (punches were sterilized with alcohol in between samples). The Following the manufacturer's instructions, the FTA® card was cleaned by adding 200 µl of Whatman FTA® purification reagent and let to stand for five minutes at room temperature.

Three times, this washing process was carried out. DNA collection cards coated with FTATM can be used to collect, store, and process biological material that has been put in a sterile 1.5 ml microcentrifuge tube; the collection tube that contained the filtrate was thrown away. After carefully opening the QI Amp Mini spin column, 100–200 µl of distilled water or Buffer TAE was added. After one minute of incubation at ambient temperature (15–25°C), the tube was centrifuged for one minute at 6000 x g (8000 rpm). It was now possible to use pure liquid DNA in solution for many studies rather than on FTA paper. This technique produced amplifiable DNA by extracting DNA in liquid form.[11]

Figure 06: FTA Card containing a blood sample, isolated DNA preserved for several days for further DNA analysis.[12]

DNA Quantitation

Agarose Gel Electrophoresis (AGE)

To determine the amount of DNA sample is most crucial for polymerase chain reaction (PCR) because narrow range works best with multiplex short tandem repeats. A well- known method was developed in 1930s as to quantify DNA samples named as Agarose Gel Electrophoresis (AGE). [13]

The term electrophoresis refers to the flow of a charged particle in an electrical field through a matrix, whereas electrophoretic mobility is dependent on both molecule radius and net charge. Agarose powder, TAE (Tris-acetate-EDTA) buffer, DNA samples, loading dye, Electrophoresis chamber, Gel comb, Gel tray, Power supply, DNA ladder, UV transilluminator, and Gel documentation system are the materials required to perform electrophoresis. Determine how much agarose powder is needed by measuring the appropriate gel percentage, which is typically between 0.7% and 2%. In a flask, combine the agarose powder and the recommended amount of TAE buffer. Heat the mixture on a hot plate or in a microwave until the agarose dissolves completely. Before putting the agarose solution into the gel tray, let it cool to around 60°C. To create wells for loading samples, place the gel comb into the agarose solution and allow it to solidify. Add the proper amount of loading dye to each DNA sample. During electrophoresis, the loading dye aids in sample loading and offers color to track migration. To guarantee adequate mixing, mix the samples gently and centrifuge them for a short time. Samples are placed on ice after being heated to 95°C for five minutes. The DNA strands are guaranteed to be completely separated by this denaturation step. Carefully remove the gel comb, leaving wells in the formed agarose gel. Place the gel tray into the electrophoresis chamber and fill the chamber with TAE buffer until the gel is totally submerged. Fill one well with the DNA ladder, then the other wells with your DNA samples. After connecting the electrodes to the power source, adjust the voltage to the desired level. Turn on the power supply and run the electrophoresis until the dye front reaches the desired distance. The voltage and time will vary depending on the size of the DNA fragments and the gel percentage. Keep an eye on the migration process to make sure the samples are separating efficiently. Carefully remove the gel from the chamber after staining it with a DNA- binding dye like ethidium bromide. View the DNA bands on the gel by placing it under a UV transilluminator. Record the results using a gel documentation system. [14]

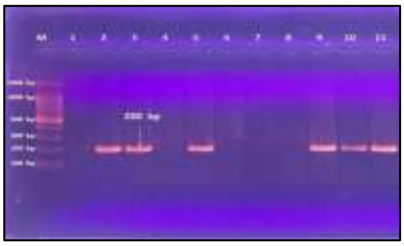

Figure 07: PCR results from extracted total DNA from the bacterial isolates under investigation using the primer papC gene and product (200 bp) were electrophoresed on an agarose gel stained with ethidium bromide.DNA molecular size marker (100 bp ladder), lane (L), and electrophoresis were conducted at 70 volts for 90 minutes. Acinetobacter lwoffii and Aeromonas salmonicida in Lane Display Positive Results with Gene papC.[15]

Slot Blot Quantitation method

In slot blots, genomic DNA was first captured on a nylon membrane, and then a human- specific probe was added. Next, the intensities of colorimetric or chemiluminescent signals were compared between a set of standards and the specimens. The slot blot technique, like nearly all DNA quantification techniques, used a relative measurement to compare unidentified samples to a set of standards. These reference samples were made by serially diluting a DNA sample with a defined concentration. The analyst's subjectivity was influenced since the comparison between the unknowns and standards was typically done visually.

Exhumation

The term "exhumation" is derived from the Latin, ex refers to "out of Humus, earth or ground". Exhumation defines as to bring to take graved corpse out of the earth, particularly following a period of burial or darkness. It is a dig, disinterment, or medicolegal emerging from a grave. In India, exhumation is uncommon as cremation, or burning, is the Hindu method of disposing of remains. Exhumation is only important to some societies, such as Muslims and Christians, who bury their dead. It turns into required if the deceased was buried without a post-mortem and there were no suspicions at the time of death. New information may later surface that demonstrates some wrongdoing. To determine the reason of his death, a post-mortem examination is therefore required. When the initial post-mortem was deemed insufficient and it is believed that a second post-mortem may reveal additional information, exhumation also becomes necessary. This essentially entails both exhumation and a second post-mortem. [16]

Another term widely used in the field of medico-legal aspect is known as excavation, refers as to the elimination of other concealed evidence like drugs, firearms items linked to human remains such as ballistic materials, ligatures, apparel, and personal belongings. Under the provision of Section 176(3) of the Code of Criminal Procedure, 1973 states that "Whenever such magistrate considers it expedient to examine the dead body of any person who has been already interred, to discover the cause of his death, the Magistrate may cause the body to be disinterred and examined." But according to Bhartiya Nagarik Suraksha Sanhita, 2023 a new Section 196(4) was created in which Section 176(3) of Code of Criminal Procedure (CrPC)is well mentioned as inquiry done by Magistrate into cause of death. [17]

There are several reasons which arises to proceed into exhumation, as when suspicion spring up about the cause of death of a deceased individual whose body has been buried. The digging up or disinterment can be performed in circumstances as in criminal cases where homicide, suspected homicide, disguised suicide, suspected poisoning, or death from illegal abortions and malpractices are the causes of death; in civil cases, Mathur argues that laws pertaining to the exhumation of bodies are necessary in cases involving unidentified bodies, workmen's compensation claims, insurance claims, and inheritance claims in which the identification of the deceased is contested. The procedure initiates after the approval of magistrate in the above-mentioned criminal cases. After the burial of dead body, there is no time upper limit as to proceed down for exhumation in countries like India and England as well. Meanwhile the countries like France have a time limit of 10 years, whereas in Germany it is 30 years. However, in Scotland maximum time to conduct exhumation until 20 years from the time of burial. As there is no time limitation on the process of exhumation arising more difficulty as to justify the commission of crime in further trials, and somehow it also causes lethal harm in health of society and defeats the morality of society as well. According to Indian Constitution, Article 21 guarantees to its citizen the Right to Life fair treatment and dignity extend not only to a living person but also to his dead body. [18]

Time of Exhumation is typically carried out during the day. The exhumation crew should thus arrive at the graveyard or burial site early in the morning. The doctor and the magistrate or coroner who ordered the exhumation should be on site. Identifying the grave with the assistance of family members and the graveyard authority, the burial is correctly recognized. The process of separating the area. The area should be screened off if there are too many interested onlookers. Soil removal from the grave is then requested to be done by professional diggers. When the coffin is seen, it is raised by passing sturdy ropes underneath it.

Exhumation is the procedure of removing a deceased person's body that has already been buried by excavating in accordance with local laws. Since most exhumations are handled by non-forensic personnel, there is a lack of a legal process for exhumation and careful study of the deceased, and there is no literature on the subject. Goal: To investigate the exhumation procedure, digging duration, burial depth, body posture, cause of death of human remains, and period between death and burial. Materials and Procedures: 18 exhumation cases from 2003 to 2015 that took place in the Vijayapura district were examined. Findings: Average burial depth of 3.56 feet, average digging time of 56 minutes, position of the corpse in 50% sitting and in 50% horizontal, in all instances expect one case skeletal remains were inspected, cause of death determined in 88.88% cases, average time of burial 103 days. Rate of decomposition is influenced by the time between death and burial. The reason of death in two instances is not discovered; thus, dirt and degraded soft tissues were kept intact. It took two to three months for skeletonization to take place. Conclusions: Regardless of the length of burial, exhumation is not always a pointless procedure, and the reason of death is typically identified. Another crucial element that influences the rate of death is the period between the date and time of death and the date and time of burial. [19]

Case of 12-year-old Missing Girl

There was a girl who vanished for almost two years. The defendants acknowledged that they had committed sexual assault, burying the victim after strangling them in order to avoid being discovered later. Following the independent identification of the burial location by the three accused, the body was removed from the earth. A big jute bag (bora) was used to dispose of the body. The body was moved to Lady Hardinge Medical College's mortuary for an autopsy following exhumation. The victim's personal items were found alongside the body, including a silver necklace, black thread with marble stone (tabeez), and, most notably, a piece of off-white net material and a little piece of off-white cloth. There were two of each of the humerus, ulna, radius, femur, tibia, and fibula among the long bones, along with their upper and lower One patella and sections that are separated. There were three sacral sections and two hip bones that were divided in half. A talus, two calcanei, and a few loose fragments of metatarsals and metacarpals were also found. Twelve teeth were missing, six in the mandible and six in the maxilla. The bones were dry, free of cartilage and soft tissues, and neither heavy nor oily. Their cranium was cracked and brittle. The ends of other bones were split. Secondary sites of ossification in the tibia, fibula, radius, ulna, and humerus at the upper and although not fused, lower ends have emerged. There is no secondary center of lesser trochanter ossification in the femur. Following a careful analysis of the skeletal remains, it was determined that the bones were those of a human female who was between the ages of 10 and 12 and who was between 138 and 140 cm tall. Common poisoning was not detected in the soft tissues that were identified with the bones. The girl's identification was verified by femur, tooth, and skull DNA analysis.[20]

Case of Izium Mass Grave

Following its recovery by Ukrainian forces during the Russian invasion of Ukraine, a number of mass graves, including one site with at least 440 victims, were discovered in woodlands close to the Ukrainian city of Izium on September 15, 2022. The bodies of those murdered by Russian forces were interred in the graves. More than 1,000 civilians are thought to have died during the conflict for and subsequent Russian annexation of Izium, according to the Ukrainian government. Ukrainian investigators report that 447 dead were found at one of the locations, including 22 troops and 414 civilian bodies (215 women, 194 men, and 5 children).

Others may have perished from bombardment and a lack of access to medical care, but the majority of the dead displayed signs of violent death, and 30 had evidence of torture and summary execution, including ropes around their necks, bound hands, shattered limbs, and genital amputation. According to Ukrainian President Volodymyr Zelenskyy, two additional mass graves "with hundreds of people" had been discovered on September 26.[21]

Challenges Arise in Exhumation in India and DNA Analysis from Buried Human Remains

Exhumation in India faces several challenges due to legal, cultural, and procedural complexities, these can include the various points such as, legal restrictions, exhumation requires authorization from a magistrate under Section 176 of the Criminal Procedure Code (CrPC), making it a strictly regulated process. Cultural sensitivities, since cremation is the predominant practice among Hindus, exhumation is rare and often limited to communities that bury their dead. Environmental factors, waterlogged graves or geological conditions can complicate the retrieval of samples and preservation of evidence. Privacy concerns, exhumation must be conducted discreetly, often early in the morning, to maintain privacy and avoid public interference. Forensic challenges, decomposition and contamination of remains can hinder forensic analysis, especially in cases involving poisoning or other chemical traces. Procedural delays, the process involves coordination among police, medical officers, and cemetery authorities, which can lead to delays. [22,23]

DNA analysis of buried human remains presents significant challenges, particularly in a country like India where environmental, technical, and procedural limitations often complicate forensic investigations. One of the foremost challenges is the degradation of DNA over time due to environmental factors. India's hot, humid, and monsoon-prone climate accelerates the decomposition of biological material. Soil conditions, microbial activity, moisture, and temperature fluctuations can all contribute to the breakdown of DNA, making it difficult or even impossible to retrieve viable genetic material from buried remains, especially if the body has been interred for an extended period.[24]

Another major challenge is the quality and availability of forensic infrastructure. Many regions lack access to advanced DNA testing laboratories equipped to handle highly degraded samples. Even where facilities exist, they may suffer from inadequate funding, equipment shortages, or understaffing, resulting in long delays and questionable reliability. In rural or remote areas, the lack of trained personnel to properly exhume and preserve samples further reduces the chances of successful DNA extraction. Contamination during exhumation or transport is another serious issue, as mishandling of remains or lack of proper protective measures can lead to sample degradation or mix-ups, which compromise the integrity of the analysis.

Legal and procedural hurdles also contribute to the complexity of conducting DNA analysis on buried remains. Obtaining court orders for exhumation, ensuring chain of custody, and securing family consent for comparative DNA samples can delay the process, further diminishing the quality of genetic material. In cases where relatives are unavailable or unwilling to provide samples for comparison, establishing identity becomes even more difficult. Furthermore, India lacks a comprehensive national DNA database, which limits the ability to match unidentified remains with missing persons efficiently. Cultural and religious sensitivities also play a role, as families may resist exhumation or DNA testing on moral or spiritual grounds, particularly when the burial occurred long ago. Lastly, financial constraints often hinder access to high-quality DNA analysis, especially in cases involving marginalized communities or unidentified individuals. Addressing these challenges requires a holistic strategy involving better funding for forensic science, training of personnel, legal reforms to streamline procedures, and public education to improve cooperation with investigative processes. Without these improvements, the potential of DNA analysis in buried human remains cases remains significantly underutilized.[25]

Mitigation of Challenges in DNA Analysis from Buried Human Remains

Mitigating challenges in DNA analysis from buried human remains involves several strategies aimed at preserving, recovering, and analysing degraded genetic material. Burial environments typically expose remains to moisture, microbial activity, fluctuating temperatures, and varying pH levels, all of which accelerate DNA degradation. To counter these effects, proper excavation protocols are essential. Forensic teams must handle remains with gloves, avoid contamination, and store samples in sterile, dry containers. Prioritizing dense skeletal elements like teeth or the petrous portion of the temporal bone can significantly improve DNA yield, as these structures offer protection against environmental damage. Once recovered, specialized laboratory techniques are employed to extract and amplify DNA. These include the use of silica-based purification methods, enzymatic repair kits to restore fragmented DNA, and next-generation sequencing (NGS), which can retrieve information from highly degraded samples.

Additionally, mitochondrial DNA (mtDNA) analysis is often favored over nuclear DNA in such contexts because mtDNA is more abundant and resilient. Bioinformatics tools are also critical, helping researchers distinguish between authentic ancient DNA and modern contamination. Environmental DNA (eDNA) from soil samples surrounding the remains may also be examined to provide context or additional genetic information. Moreover, maintaining strict chain-of-custody procedures and including negative controls during lab work help to mitigate contamination and ensure reliable results. In mass grave investigations or archaeological contexts, collaboration between archaeologists, anthropologists, and geneticists enhances the overall success of DNA recovery by aligning excavation methods with genetic analysis requirements. Ultimately, while DNA from buried remains is often compromised, advances in technology and interdisciplinary approaches significantly improve the potential for successful identification and genetic profiling, contributing to forensic investigations, historical reconstructions, and humanitarian efforts.[26]

Application of Exhumation in Forensic Science

Sometimes, exhumations are essential to forensic investigations in order to reveal information that was not readily available or obvious during the original inspection. The significance of exhumation in ensuring safety, justice, and resolution is highlighted by each of these factors. It is still our top responsibility to handle these matters professionally and sensitively, making sure that all legal requirements are met and that families are taken into consideration during our processes. The unresolved cases involving new techniques have been developed to more precisely determine the causes of death in instances that have not been resolved, occasionally as a result of technological changes. This can be important in criminal situations because the verdict could have a big impact on the result. Environmental scientists and public health officials can evaluate the threat and stop wider exposure by exhuming the remains of a corpse if there is a possibility that it was exposed to dangerous substances or materials. Gathering out more evidences, in any instances where the first autopsy was inconclusive, or when foul play is subsequently suspected, exhuming the body permits forensic professionals to do deeper studies, potentially with current equipment or procedures that weren't originally applied. Families who are looking for answers regarding their lost loved ones may find closure if exhumation is necessary to identify the identification of a deceased person in unfortunate situations when their body was buried unrecognized due to subsequent discoveries or advancements in DNA technology.[27]

Exhumation is a regular practice in the investigation of forensic scenes when bodies have been hidden by burial, and it is occasionally done from official, marked graves during the investigation of suspicious deaths. In the latter situations, the retrieval of the bones as well as related physical evidence (such as artifacts, dirt, and insects) can help investigators ascertain the cause and date of death. Executed victims' exhumations from mass graves have yielded crucial evidence for the prosecution of those charged with crimes against humanity and war crimes. [28,29]

Review Of Literature

Gino S., et. al. (2003), conducted a research experiment on DNA typing from epiglottic cartilage of exhumed bodies, performed experiment on the three exhumed bodies of 6, 7 and 18 months, showing presence of well-located matrix delimiting the lacunae, which locating out the residues of chondrocytes. DNA extracted from the tissue is 0.25 ng/mg considering for the 6- and 18-months exhumed bodies and 2 ng/mg of tissue from body exhumed after 7 months, by using organic phenol- chloroform method and quantified using Quantiblot Human DNA Quantification Kit. GeneScan™ software was applied to analyze the electrophoretic data. The automatic genotyping of DNA samples was performed by Genetyper 2.5 software in alliance with AmpFISTR Cofiler Template file. This research experiment showed that when decomposed human remains were analyzed the alternative source of genomic DNA can be DNA extracted from epiglottic cartilage as to identify and also perform paternity testing as well. [30]

Sahib Z. M., et. al. (2013), conducted the experiment over 24 human old skeletal remains which were collected from Pakistan mass graves 200-500 years old for DNA typing. Each and all bones were grinded into small fragments using a dental diamond disk and radiated against ultra violet for 10-15 mins. The bone fragments are treated with 10% bleach and 95% of ethanol to remove the down remaining contamination of soil, dirt and other debris. Liquid nitrogen and SPEX 6750 Freeze/Mill (SPEX CertiPrep, Metuchen, NJ) and were stored in a 15mL of falcon tube and stored at -20 degree C till the DNA extraction performed. QIAquick PCR purification kit (Qiagen) was used to extract out the DNA sample and Quantifiler Duo Human DNA Quantification kit and ABI Prism 7500 Real Time PCR System were used to quantify DNA samples. The thermal cycling was conducted on PTC-200 (Peltier Thermal Cycler) DNA engine used to amplify DNA samples, which were kept 28, 32 and 33 during all experiment. The DNA samples were injected on ABI Prism 3130 Genetic Analyser (ABI) and a data analyser named GeneMapper ID software version 3.2 was used to analyse STR loci. Almost 17 samples were detected under Real-time PCR quantification, while 7 samples could not. They interpreted the result after analysis of 24 highly degraded samples from old skeletal remains, as of 9 full DNA profiles and 11 partial DNA profiles, but 4 samples were obtained with no profiles. [31]

Kumar T. M., et. al. (2019), conducted a research experiment on two different types of tissue samples (includes brain cortex and uterus of females/ prostate gland of males) and samples of teeth were collected from 25 days of post mortem interval of 20 unclaimed decomposed bodies. The tissue samples were collected from All India Institute of Medical Science (AIIMS), New Delhi and stored within sterilized plastic containers at − 80 °C. Phenol-chloroform method was used to extract out the DNA from tissue samples and stored at −20°C the extracted DNA sample were quantified by using Quantifiler Trio DNA Quantification kit and an ABI Prism 7500 detection system. The extracted and quantified DNA samples were amplified with Promega PowerPlex 21™ STR kit and genotyped with the help of capillary electrophoresis in 3500 XL Genetic Analyzer. They came to a conclusion that the success rate of samples of brain is found better than the samples of prostate/ uterus. A profile of all 21 STR markers in 16 and 9 samples were given by the brain and teeth samples, respectively. But a partial amplification was developed by prostate gland and no prostate sample could be able to yield a complete profile.[32]

Turingan S. Rosemary, et. al. (2019), research conducted on rapid DNA analysis technique in identification of human remains. They performed an experiment using ANDE in Forensic Anthropology Centre (FAC) at University of Tennessee over 10 deceased human remains which includes 7males and 3 females. They collected 2 brain samples (BD01 and BD02), 8 liver samples (LD01- LD08) and 7 teeth samples, which were stored immediately at -20degree C. Initiating the process of rapid DNA in two different types of chips, I- chips for Unidentified Human Remains (UHR) and A- chips for Family Reference Sample (FRS). For creating DNA ID that contained all 27 FlexPlex™ loci, by processing the samples using both PowerPlex®Fusion 6C and PowerPlex® 21 (Promega, Madison,WI). Approximately 94 min 5 samples in A- chip processes in a single run, and around 103 min 4 samples in I- chip. As a result, they concluded that DNA IDs were all reporting as either full (all loci amplified; 27 males and 24 females), partial or background (no alleles called). There were no discrepancies in the DNA IDs were found between liver, brain, bone, and tooth. [33]

Liu Zhiyong et. al. (2020), conducted a research experiment based on the DNA typing from skeletal remains by using Capillary Electrophoresis (CE) and Massively Parallel Sequencing (MPS). They performed this experiment over 35 human skeletal samples (9 females and 26 males) ranging from 3- 15 years, which was collected from the Institute of Criminal Science and Technology of Hangzhou Public Security Bureau. 35 skeletons (including 4 canines and 5 molars) were scraped with surgical operation knife. Skeletons cleaned by deionized water and 2 hours of UV exposure to reduce contamination. Every tooths was 75% aquas ether for 10 mins and deionized water used and UV exposure. The samples were treated with liquid nitrogen grinding method. PrepFiler™ BTA Lysis Buffer was used and centrifuged at 14000 rpm for 3 mins, to extract out the DNA sample. the quantification of DNA sample was completed by Quantifiler™ Trio DNA Quantification Kit. These samples were analyzed using capillary electrophoresis and massively parallel sequencing, resulting 124 further SNPs were genotyped using the MPS platform. Overall, STR markers based on MPS are superior to CE. STR markers in order to determine the quantity of loci and sub-alleles. Our research shown that MPS was a more effective method of detecting more STR and SNP, resolving issues with degraded samples that were not addressed by conventional CE-based STR. [34]

Discussion

DNA extraction and analysis from buried human remains is a vital process in forensic science, archaeology, and anthropology, offering a window into both individual identification and broader population history. The recovery of DNA from decomposed or ancient remains poses significant challenges due to the degradation of genetic material over time. Environmental factors such as temperature fluctuations, soil composition, microbial activity, and moisture levels can severely affect the preservation of DNA. However, advancements in molecular techniques have made it increasingly possible to extract usable DNA even from highly compromised samples. The process typically involves careful sampling from dense bone tissues like the petrous part of the temporal bone or teeth, which offer better protection against environmental damage. Strict contamination control measures are essential, as even minute traces of modern DNA can compromise results. Once extracted, DNA can be amplified using polymerase chain reaction (PCR) or analyzed through next-generation sequencing (NGS), which allows for the study of both mitochondrial and nuclear DNA.[36]

Applications of this analysis are vast. In forensic investigations, it can help identify unknown victims, resolve cold cases, or establish familial relationships. In archaeology and bioanthropology, DNA analysis provides insights into ancestry, migration patterns, population genetics, and ancient diseases. However, the interpretation of ancient DNA (aDNA) must be approached with caution, considering the potential for contamination and the fragmentary nature of the genetic material. Ethical considerations are also central to the process. The extraction and study of human remains must respect the cultural and spiritual beliefs of descendant communities, and in many cases, require permissions or collaborations with local authorities.

Findings

The study and practice of DNA extraction from buried human remains have yielded significant scientific findings, transforming both forensic and archaeological investigations. One of the most important discoveries is that, despite prolonged burial and exposure to harsh environmental conditions, recoverable DNA can still be present in skeletal material, particularly in dense bone structures like teeth and the petrous part of the temporal bone. These structures often provide the highest DNA yields due to their protection from microbial degradation and environmental exposure. Research has shown that environmental factors such as soil acidity, temperature, humidity, and microbial activity greatly influence the preservation of DNA in buried remains. For instance, cold and dry conditions are more favorable for DNA preservation compared to warm, humid, or acidic environments. Consequently, DNA retrieval success rates vary significantly based on burial context.

Advances in molecular biology have led to improved protocols for the extraction of both nuclear and mitochondrial DNA from degraded samples. Mitochondrial DNA (mtDNA), due to its higher copy number per cell and greater durability, is frequently used in cases where nuclear DNA is too degraded. The development of next-generation sequencing (NGS) and improved PCR techniques has made it possible to analyze even highly fragmented DNA, enabling the reconstruction of genetic profiles from remains that are hundreds or even thousands of years old. In forensic science, these findings have been pivotal in identifying unknown individuals from mass graves, historical conflict zones, and cold cases. For example, DNA analysis has helped identify victims of the 9/11 attacks, the Balkan wars, and the Holocaust. In archaeology, ancient DNA (aDNA) studies have provided insights into human evolution, migration patterns, familial relationships, and even the spread of diseases.[36]

Another critical finding is the importance of contamination control. Since ancient and degraded DNA is often present in very small quantities, rigorous laboratory protocols are necessary to prevent contamination from modern DNA, which could otherwise compromise the results. Overall, findings from this field have demonstrated that DNA analysis of buried human remains is not only feasible but highly informative. It plays a crucial role in both scientific discovery and humanitarian efforts, offering a powerful means to connect past and present through the genetic record preserved in human remains.[37]

Conclusion

The extraction and analysis of DNA from buried human remains represent a vital intersection of science, history, and justice. Despite the challenges associated with degradation, contamination, and environmental interference, modern advancements in molecular biology and forensic science have significantly enhanced our ability to retrieve and interpret genetic material from aged and compromised samples. These developments not only aid in the identification of individuals from archaeological, historical, and forensic contexts but also contribute to a broader understanding of human ancestry, population migrations, and cultural practices. One of the key challenges in working with buried remains is the degradation of DNA over time due to factors such as microbial activity, temperature fluctuations, soil acidity, and moisture. These factors often lead to fragmentation and chemical modifications in DNA, making it difficult to retrieve intact genetic material. However, techniques such as next-generation sequencing (NGS), polymerase chain reaction (PCR) amplification of short target regions, and improved protocols for decontamination and preservation have made it increasingly possible to overcome these obstacles. The use of mitochondrial DNA (mtDNA), due to its higher copy number and resilience, is particularly valuable in cases where nuclear DNA is too degraded to analyze effectively.

The ethical dimensions of working with human remains are also critical to consider. Researchers must balance the scientific value of such investigations with respect for the deceased and the cultural sensitivities of descendant communities. Transparent methodologies, clear permissions, and collaboration with relevant stakeholders are essential components of ethical DNA analysis in both forensic and archaeological settings. Forensic applications of DNA analysis from buried remains are particularly impactful in identifying victims of crime, war, genocide, and natural disasters. Such work provides closure to families and plays a crucial role in legal processes. In the realm of archaeology and anthropology, ancient DNA (aDNA) has revolutionized our understanding of past human populations, shedding light on migration patterns, disease prevalence, and kinship structures. Moreover, DNA analysis has enabled the reassessment of historical narratives and provided a genetic link between past populations and present-day communities.

Looking ahead, the integration of emerging technologies such as artificial intelligence, improved bioinformatics tools, and portable DNA sequencing devices promises to make DNA analysis more efficient, accurate, and accessible. These advancements may further minimize the amount of sample material required, reduce contamination risks, and open new possibilities for in-field analysis. In conclusion, DNA extraction and analysis from buried human remains is a powerful tool that bridges the biological and historical sciences with forensic investigation. It serves as a gateway to the past and a key to justice in the present. As techniques continue to evolve, so too does our ability to uncover, interpret, and honor the stories encoded in human remains, reinforcing the value of interdisciplinary collaboration in unlocking the genetic legacy of humankind.

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