Case Report
Volume 16:2, 2025

Journal of Forensic Research

ISSN: 2157-7145 Open Access

DNA Fingerprinting: Identification of the Individuals in Distressing Disaster Situations-Case Report

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Abstract

Advancement in technology, safety in Indian railways has improved which decline the rates of train accidents *i.e.*, collisions, derailment etc. However, train accident at Bahanga railway station at Balasore occurred on 2nd June 2023 in which around 293 passengers lost their lives and nearly 1200 passengers were injured raise question on railway safety measures. The main objective in such disaster is to identify the victims beside the reason behind the mishap.

Forensic DNA analysis plays a key role in identification of mutilated bodies. As many as 81 deceased could not be identified mainly due to mutilation as many changes were observed in their bodies in due course of time; some were decapitated, in others only long bones were available. Also, most of these were not identified as they were travelling in unreserved compartments where there was no record available with the Railway Division. Sternum, teeth, scalp hairs and long bones which ever found suitable for DNA analysis were collected.

The DNA analysis using autosomal STRs and Y-STRs was carried out for these unidentified bodies. As these bodies were embalmed using 10%-40% formalin for preservation so it is difficult to generate DNA profile even though DNA profile was generated successfully from all these 81 unknown bodies (100%) after 8-10 days of accident and compared with the claimants. 53 bodies were identified and handed over to their relatives on the basis of DNA Fingerprinting match, rest 28 bodies were disposed of. Sternum emerged as ideal sample for identification in comparison of teeth and long bones even after exposed to 40% formalin.

Keywords: Disaster management • Forensic DNA identification • Train collisions • Sternum bone • Teeth

Introduction

Forensic DNA testing has a number of applications, including civil disputes such as parentage testing, identifying human remains from natural or man-made disasters and solving crimes such as homicidal assaults, decapitation, motor vehicular accidents involving facial deformities in crush in uries, terrorist attacks etc. This technique has a valuable role in the field of forensic Science and has emerged as a powerful tool to generate crucial leads to identify unknown human remains [1]. This is currently considered as the gold standard for identifying mass disaster victims. DNA analysis is the main method of choice to identify individual mass disaster victims from severely dismantled, fragmented and commingled bodies or severely charred, decomposed or skeletonized bodies. Identification of human remains is not only required in medico-legal cases or in forensic situations but also in many other situations like Human Right's context, death of migrant, mass fatality deaths etc. [2,3].

STR (Short Tandem Repeat) analysis is carried out universally for all forensic purposes and the number of STR loci validated for forensic use has now grown to at least 27 loci. Worldwide variations of allele frequencies at these loci have been studied, showing that variations of inter-population diversity at these loci do not compromise

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Received: 21 February, 2024, Manuscript No. JFR-24-127991; **Editor assigned:** 26 February, 2024, PreQC No. JFR-24-127991 (PQ); **Reviewed:** 12 March, 2024, QC No. JFR-24-127991; **Revised:** 14 April, 2025, Manuscript No. JFR-24-127991 (R); **Published:** 21 April, 2025, DOI: 10.37421/2157-7145.2024.16.651

the power of identification of individuals. However, data collected for validation of these loci for forensic use has utility beyond human identification; the origin and past migration history of modern humans can be reconstructed from worldwide variations at these loci. Furthermore, complex forensic cases which remain previously as unresolved can now be re-investigated with the help of the validated STR loci [4].

New technologies and commercial ready-to-use kits have made DNA analysis possible and can be carried out in short duration. Also, modern technology has enabled to carry out DNA analysis even with a very minute quantities of available DNA within the samples recovered. During DNA-based identification process, utmost care has to be taken to collect and preserve body tissues for DNA extraction as fast as possible, since DNA begins to degrade since the time of death and this degradation can be accelerated by environmental factors especially humidity, temperature and microbial activity.

The personnel collecting the body tissue for DNA analysis at a later stage should have thorough knowledge about the sampling source or tissue to be collected, depending upon the state of the decomposition and environmental factors. Multi-rooted teeth, long bones such femur and red skeletal muscle represent the best source for DNA sampling.

Care should be taken to immediately preserve the collected DNA samples with available preservation methods and the collected samples should be processed as soon as possible. Utmost care should be taken not to contaminate the DNA samples. Reference family samples should be preferably taken from victim's parents or siblings of the victims whenever possible and feasible.

DNA analysis method of identification method is reliable and far superior to the other conventional methods used for identification of an individual. With ever increasing DNA databases of Government of various countries, it is recommended in near-future to develop a point of care chip to rapidly conduct DNA analysis and matching. Hence DNA analysis should be considered and planned in every mass disaster incident. DNA analysis should be carried out on all the victim body and fragments regardless of the body being initially identified by other preliminary modes of identification. It can be safely concluded that DNA analysis has revolutionized and has significantly aided in the identification of mass-disaster victims.

Railway accidents across the world are reported quite few nowadays. These tragic mishaps could occur due to several reasons: Inevitable natural calamities such as floods, earth quakes or man made errors like signal delays, miscommunication, operational mismanagement, system failure, machinery fault, maintenance flaws or sabotage etc. The multi-disciplinary involvement of investigating agency including Forensic experts in such distressing situations is of utmost help to lead the investigation in efficient and proper direction. The primary job apart from rescue operation is not only to find the root cause behind this mishap but also to elicit the identity of victims.

The procedure adopted in India for the identification of the victims in such disastrous situations is generally based on the recognition of morphological features, personal belongings e.g., clothing's, jewelry, mobile phones, aadhaar cards or other identity cards by the relatives or next-of-kins. However, these methods become unsuccessful and highly difficult when the bodies were beyond recognition. Though in some developed countries antemortem dental records have been extensively used for the identification purpose but such data is not usually available in developing countries like India. Thus it has been suggested that DNA based identification would be more effective and efficient in handling situations like this [5,6].

An unfortunate train accident took place at Balsore district in the state of Odisha, Eastern India on 2nd June 2023 and it was reported three trains collided, while the Coromandel Express entered the passing loop instead of the main line near Bahanaga Bazar railway station with high speed and collided with a stationary goods train. Around 293 passengers lost their lives and as many as nearly 1200 passengers were injured.

Rescue teams mobilized the injured ones to the nearest hospitals for treatment and bodies of the many passengers who succumbed to death were handed over to their family members after matching reservation coach list and seat alignment and fulfilling legal formalities. However, eighty-one deceased bodies could not be identified either due to decomposition changes and severe mutilation near decapitation etc., in due course of time where only remnants such as long bones were available. The difficulty in identification raised because the details of those individuals travelling in unreserved compartments was not traceable in railway records available with the railway division.

Keeping in mind about the accuracy of this technology it was decided to identify the 81 deceased using DNA analyses so that these bodies are handed over to the right families and so that their last rites are performed accordingly with dignity. Also, Government bodies announced ex-gratia as supplementary relief to the next-of-kin and the money was supposed to be disbursed to the correct family member.

Case Presentation

An unfortunate train accident took place at Bahanga railway station at Balasore district in the state of Odisha, Eastern India on 2nd June 2023 and it was reported around 293 passengers lost their lives and nearly 1200 passengers were injured.

No data are available regarding the complete success of DNA Short Tandem Repeat (STR) profiling from degraded human remains pertaining to train accidents in India till date. Though Madhusudan, et al., [7] carried out the study on the victims of Mangalore Air crash in 2010 where all the victims were not identified, using DNA Fingerprinting technology.

In the present disaster, Sternum, teeth, scalp hair and long bones which ever found suitable for DNA analysis were collected. The DNA Fingerprinting analysis using autosomal STRs and Y-STRs was carried out for these unidentified bodies which were mutilated and mangled beyond recognition. The bodies were segregated in four batches and embalmed using 10%-40% formalin for better preservation till further process. Though extraction of DNA is laborious and difficult in already embalmed bodies, in current scenario, DNA profile was generated successfully from all these 81 unknown bodies (100%) after 8-10 days of accident which were subjected for matching with the claimants. 53 bodies were handed over to the relatives on the basis of DNA fingerprinting positive match, rest 28 bodies were disposed of. Out of all four exhibits preserved for DNA analysis sternum serves as reliable biological sample even after exposed to 40% formalin.

Methods employed

The total numbers of unclaimed deceased bodies were 81 in number and segregated in 4 batches. Each body was embalmed with 10%-40% formalin depending on their hospital policy. Some of the bodies were kept in cold storage and others were kept in temporary cold storage carriers, connected to the generators to supply electric power without disturbance.

The sternum, teeth, long bones and hair samples were collected from these deceased where ever they were intact. These biological samples were contaminated to some extent as the bodies were lying on railway track for some hours before shifting them to cold storages. The biological samples were cleaned using distilled water, dried immediately there itself using sterile filter paper and then placed in plastic containers with proper labeling and sealing. These samples were transported to the forensic DNA lab (JPNATC, AIIMS, New Delhi) where they were processed further taking all precautions regarding handling of the said samples. Since the sternums were already cleaned off the muscles tissue while eviscerating from the body, they were cut along the axial plane to expose the underlying bone marrow which was removed (nearly 1 µg) for further process (Figure 1).

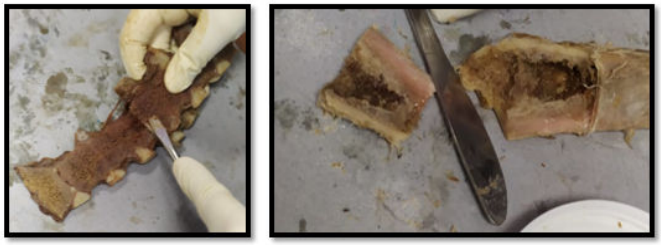

Figure 1. Collection of bone marrow.

Similarly, 3-4 teeth were extracted using molar extractors from most of the deceased bodies and cleared off the dirt at the collection site and processed in the laboratory to remove tooth pulp using instruments (Figure 2). Proximal end of hairs (Figure 3) were cut and washed properly to remove any dirt which may adhere during collection of hair from the dead body. Long bones were also cut transversely to get bone marrow. The samples were processed in EZ1 for isolation of DNA. This isolation process took nearly 2 hrs. It is based on magnetic beads particle technology. This technology combines the speed and efficiency of silica based DNA purification with the convenient handling of magnetic. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted either in the water or TE buffer.

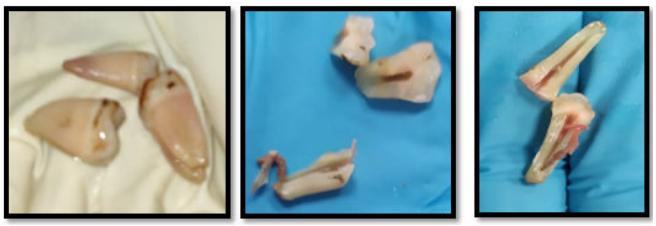

Figure 2. Collection of tooth pulp from teeth.

Figure 3. Collection of hair sample.

Since all these biological samples were collected from mutilated bodies, large amount of biological material along with extra Proteinase K and DTT was used. In normal course the 20 µl of a 20 mg/ml stock of Proteinase K is used but in this study around 80 µl of Proteinase K was used so as to digest many contaminating proteins which were expected in these samples to get good yield and good quality of DNA. In this case total 61 sternums, 2 long bones, 14 tooth and 4 hair samples were used for this analysis.

DNA isolated was then purified using centrifugal device and DNA concentratorand then subjected to PCR and finally genotyping was done using autosomal STRs kit and Y-STRs kit.

The fresh blood samples were collected from the claimants (so far blood sample collected from 118 claimants) either in EDTA vial or FTA cards at AIIMS Bhubaneswar and transported to AIIMS, New Delhi and then processed for DNA fingerprinting analysis

Results and Discussion

One of the deadliest train accident in India occurred on June 6, 1981 related train derailment, which had led the compartments of the train falling into the river while crossing a bridge over Bagmati in the state in Patna of Bihar state located in Northern India, killing between 500 to 800 people. The passenger train was moving from Mansi to Saharsa when seven of the train's nine cars fell into the river. The bodies were handed over to the relatives on the basis of personal identification of belongings as there was no scientific technique available those days to identify the individuality with 100% accuracy.

To identify the cause of death and timely disbursement of justice to victims and their aggrieved families in such situations is the primary motive of the police, forensic services and the judicial system. Government of India has established the Railway Protection Force (RPF) and Government Railway Police (GRP) for the protection of railway property and handling crime which takes place in their vicinity.

Among other procedures one important task was to identify the mutilated bodies which were beyond recognition and DNA Fingerprinting analysis was carried out for the bodies as it is the scientific technique to establish the identity of an individual as no two individual have same genetic makeup except in case of identical twins [8].

Though long bones are supposed to give good results but in the present case it is the sternum which gave better results. In a study by Ana Milos, et al., [9] the highest success rates were observed with samples from dense cortical bone of weight-bearing leg bones (femur 86.9%), whereas long bones of the arms showed significantly lower success (humerus 46.2%, radius 24.5%, ulna 22.8%). Intact teeth also exhibited high success rates (teeth 82.7%). DNA isolation from other skeletal elements differed considerably in success, making bone sample selection an important factor influencing success.

Sri Lanka tsunami train wreck is also one of the deadliest recorded train disasters in history, claiming the lives of at least 1,700 people. The incident was the result of a devastating tsunami caused in 2004 Indian Ocean earthquake, which caused severe destruction to railway infrastructure. Only 900 bodies were recovered rest were washed away due to Tsunami or taken by the relatives [9].

In Greece on 3rd March 2023 there was a collision of a passenger train and a freight train, it was the country's deadliest ever mishap, in this case only 24 bodies out of 60 dead were identified using DNA technique, where as in the present case DNA profiling was generated successfully from all the 81 deceased [10].

In a fire accident at Tazreen fashions garment in Bangladesh, Akhteruzzaman, et al., [11] in their article reported 112 individuals were killed, 50% were initially identified by visual recognition and other methods which did not followed any Interpol protocol for DVI. Samples were collected from tissues (n=35), bone (n=2), teeth (n=22). Out of 59 unidentified dead bodies, 43 were confirmed by DNA analysis with the help of 68 biological relatives originating from 61 families where as in present case DNA has been successfully generate from all the 81 deceased and matched with 53 claimants so far.

Skinner et al., mentioned that in between 1991-1999, in Yugoslavia conflict, out of 30,000 missing individuals, only two-third of the 15,000 victims were identified through DNA. 23% of 3919 remains were identified through dental information [12], where as in present study no dental information was recorded or used. Attack on World Trade Centre on 11th September 2001, a massive disaster, where thousands of people lost their lives.

Biesecker, et al., discussed the role of DNA typing in identification of the victims. There was a huge task for identification of almost 3000 victims. The victim remains ranged from a few nearly complete bodies to tiny fragments of charred bones. There was extensive fragmentation of remains with commingling and mixing with building materials. There were about 20,120 victim fragments. Till 2005, about 850 of the 1594 victim identifications for the 2749 victims were solely based on DNA analysis [13].

The DNA of good quality and quantity was obtained from all the biological samples belonging to 81 deceased and all the claimants. ard tissues are mostly preferred in situations like this as these are the longest surviving exhibits. All the 81 deceased were stored properly at lower temperature, dissected properly with all proper care and biological exhibits removed with due care. Bus and Allen in 2014, mentioned in their article that the faulty methods can lead to irreversible loss of data and errors in DNA analysis. They were able to identify only 26 out of 40 bodies using DNA analysis as were not identified using other conventional methods. They also stressed the importance of proper sampling techniques, storage and protection against contamination and extraction methods with respect to DNA analysis.

Conclusion

The trains collided at Balsore and around 293 people lost their precious lives and many more were in ured. Many of the deceased were identified using their identity cards or details with the railway authorities but 81 deceased could not be identified as the bodies were mutilated and were beyond recognition. The Government announced ex gratia to the families of the deceased and thus made it mandatory to identify the deceased with accuracy. Left with no choice, it was decided by the authorities to go for DNA analysis as is considered the most accurate method for the identification of anindividual.

All 81 unclaimed deceased samples have generated DNA profile successfully. The claimants deposited their fresh blood and so far 53 bodies have been matched and handed over to the families. This technique has helped a lot by sanctioning ex-gratia to the right families also despite of bidding farewell to the dear ones with honor. It is the first time when DNA profiling has been generated from all the deceased. This case showed that sternum sample act as an ideal sample in mass disasters even exposed to 40% formalin.

Conflict of Interest Statement

Declaration of competing interest the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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How to cite this article: Raina, Anupuma, Ajay Parkash, Jhansi Lakshmi Mylapalli, and Deki Palmo Bodh, et al. "DNA Fingerprinting: Identification of the Individuals in Distressing Disaster Situations-Case Report." *J Forensic Res* 16 (2025): 651.