Case Report

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Dna Profiling: Identification of Corpse in a Distressing Disaster Situation

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ABSTRACT

Safety measures in Indian railways have improved which has declined the rates of train accidents like collisions and derailment. 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 which has again raised question on railway safety measures. One of the main objectives in such disasters is to identify the victims beside the reason behind the mishap. Forensic DNA analysis plays a key role in identification of mutilated bodies as it provides proficiency in discriminating biological exhibits in forensic situation. 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, while in others only body parts were available. Also, most of these victims were not identified as they were travelling in unreserved compartments where there was no record of passengers available with the Railway Division. Sternum, teeth, scalp hairs and long bones which ever were found suitable for DNA analysis were collected. As these bodies were embalmed with 10% formalin, therefore it was a little challenging to generate DNA profile from all of them. With minor modifications in the protocol, DNA profile was generated successfully from all these 81 unknown bodies (100%) after 8-10 days of accident and compared with the claimants The DNA analysis was carried out for these unidentified bodies using commercially available autosomal STRs and Y-STRs kit. Although all exhibits were challenging but sternum emerged as ideal sample for identification in comparison to teeth and long bones even after exposed to 40% formaldehyde. 53 bodies were handed over to the relatives on the basis of DNA Fingerprinting positive match. Since there were no claimants for rest of the 28 bodies, the last rites for all these were fulfilled after following all legal formalities.

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, road traffic accidents involving facial deformities in crush injuries, and terrorist attacks. Nevertheless, 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] DNA analysis technique is known as a reliable and far superior technique to the other conventional methods used for identification of an individual. This technique is currently considered as the gold standard for identifying individuals and is the main method of choice to identify 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 rights context, death of migrant, mass fatality deaths etc.[2,3]

STR (short tandem repeat) analysis is carried out universally for all forensic identification purposes and the number of STR loci validated for forensic use has now reached up to 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 the power of identification of individuals. However, the validated data of these loci to be used for forensic purpose has been observed and has utility beyond human identification; the history of humans is possible to be reconstructed from worldwide variations at these loci. Furthermore, forensic cases which remain previously 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 quantity 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. Biological samples should be processed as soon as possible for DNA analysis taking utmost care not to contaminate the biological samples. Reference family samples should be preferably taken from victim's parents or siblings whenever possible and feasible.

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, earthquakes 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, jewelry, mobile phone, Aadhaar card or other identity cards by the relatives or next-of-kins. However, these methods become unsuccessful and highly difficult when the bodies are 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 countries like India. Thus, it is suggested that DNA based identification would be more effective and efficient in handling situations like this.[5,6]

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 and disposed of 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 families.

CASE REPORT

An unfortunate train accident took place at Balasore district in the state of Odisha, Eastern India on 2nd June 2023 and it was reported that 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 could not be identified either due to decapitation or changes due to decomposition or severe mutilation etc., in due course of time where only remnants such as long bones were available. The difficulty in identification rose, one of the reasons being that the details of those individuals travelling in unreserved compartments were not traceable in railway records available with the Railway Division.

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METHODS EMPLOYED

The total numbers of unclaimed deceased were 81 in number. Due to limited space in mortuary, some bodies were kept in temporary prepared cold storage carriers, connected to the generators to supply electric power without disturbance due to limited space in mortuary.

In the present case, the bodies were segregated in four batches according to serial numbers and embalmed using 10% formalin for better preservation till further process. 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. Sternum, teeth, scalp hair and long bones whichever found suitable for DNA analysis were collected from all these 81 unknown bodies after 8-10 days of accident which were beyond recognition. 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. They were well preserved and transported to 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 tissues while eviscerating from the body, they were cut along the axial plane to expose the underlying bone marrow which was removed (nearly 1µg) for further process. [Figure 1a & 1b]

Figure 1. Figure 1a & 1b: Collection of bone marrow

The extraction of DNA is laborious and difficult in formalin embalmed bodies, though in current scenario, the samples were challenging even then DNA profile was generated successfully from all these 81 unknown bodies (100%) and were subjected for matching with the claimants. The DNA Fingerprinting analysis using commercial autosomal STRs and Y-STRs was carried out for all these bodies. 53 bodies were handed over to the relatives on the basis of DNA Fingerprinting positive match; rest 28 bodies were cremated as there were no claimants. Out of all four exhibits preserved for DNA analysis, sternum served as the best and reliable biological sample even after exposure to 40% formalin.

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 obtain tooth pulp. [Figure 2a to 2c]. Proximal end of hairs [Figure 3] were cut and washed properly to remove any dirt which may have adhered during collection of hair from the dead body. Long bones were also cut transversely to get bone marrow (Teeth- 04, Long Bones-07, Sternum-70 were used to obtain DNA from these deceased's). The samples were processed using Qigen EZ1 Extraction machine for isolation of DNA. This isolation process took nearly 2 hrs. This method is based on magnetic beads particle technology. The DNA is then efficiently washed and eluted in the TE buffer.

Figure 2. Figure 2a to 2c: Collection of tooth pulp from teeth

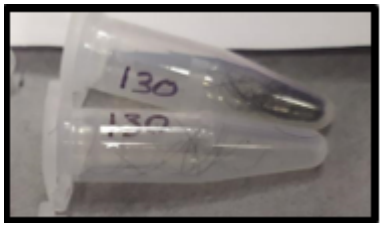

Figure 3. Collection of hair sample

Since all these biological samples were collected from mutilated bodies, large amount of biological material along with proportional amount of Proteinase K and DTT was used. In normal course, the 20µl of a 20mg/ml stock of Proteinase K is used to get good yield and good

quality of DNA. In this case, a total of 61 sternums, 2 long bones, 14 teeth and 4 hair samples were used for this analysis. DNA isolated was then purified using centrifugal device and DNA concentrator. Further, isolated DNA was 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) in EDTA vial and FTA cards at AIIMS Bhubaneswar and transported to AIIMS, New Delhi and then processed for DNA profiling.

RESULTS AND DISCUSSIONS

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

Problems are normally faced during DNA typing of degraded samples especially with low template DNA or Low copy number DNA (≤ 100-200 pg/μL) present in degraded DNA sample. More recently, Gill et al.,8 mentioned LCN as any DNA sample or DNA profile where stochastic effects are present and/or where the alleles detected are below a laboratory defined stochastic threshold which includes allele drop out, allelic drop-in, heterozygous peak height imbalance, locus drop-out and elevated stutter peaks. Though there are several approaches to analyze samples with low quantity of DNA like: using highly effective DNA extraction method, increasing sensitivity of PCR amplification and consensus approach. But consensus approach was observed as more reliable and reproducible.[9,10]

One of the deadliest train accidents in India occurred on 6 June 1981 — a train derailment that caused the compartments to fall into the river while crossing a bridge over the Bagmati River in the state of Bihar in Northern India, killing between 500 to 800 people. The passenger train was moving from Mansi to Saharsa when out of nine bogies, seven fell into the river. The bodies were handed over to the relatives on the basis of personal identification of belongings as there were no scientific techniques 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 best scientific technique to establish the identity of an individual as no two individuals have the same genetic makeup except in case of identical twins.[11]

Though long bones as reported earlier, has observed good results but in the present case it is the sternum which demonstrated better results**.** In a study by Miloš et al.,12 the highest success rates were observed with samples from dense cortical bone of weight-bearing leg bones (femur 86.9%), whereas upper long bones showed lower success (humerus 46.2%, radius 24.5%, ulna 22.8%) and intact teeth demonstrated high success rates (teeth 82.7%). The varying success rates of DNA isolation from different skeletal remains indicate that bone sample selection is important for optimising success rates.

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.

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.[13]

In a fire accident at Tazreen fashions garment in Bangladesh, Akhteruzzaman et al.,14 in their article reported 112 individuals were killed, 50% were initially identified by visual recognition and other methods that did not follow 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 generated from all 81 deceased and matched with 53 claimants so far.

Skinner et al.,15 mentioned that only two third victims were identified using DNA finger printing analysis. 23% of 3919 remains were identified through dental information, 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.,16 discussed the role of DNA typing in identification of almost 3000 victims. The victim remains ranged from a few nearly complete bodies to tiny fragments of charred bones. Whereas some bodily fragments were found commingling and mixed with building material. 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.

The DNA of good quality and quantity was obtained from all the biological samples. The most challenging condition was that all these bodies were embalmed using high amount of formalin but even then these exhibits generated DNA profiles with high accuracy. Hard tissues are mostly preferred in situations like this as these are the longest surviving exhibits.[3] All the 81 deceased were stored properly at lower temperature, dissected properly with all proper care and biological exhibits removed with due care. Although Raina et al.,17 reported partial DNA profiles (8-9/15 loci) were obtained from histopathology block of a patient diagnosed with high-grade breast carcinoma.

Buś 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 rest of the deceased were not identified even after 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.[18]

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. Therefore, DNA analysis should be planned in mass disaster incidents and should be carried out on all the victim bodies 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.

CONCLUSION

The trains collided at Balasore and around 293 people lost their precious lives and many more were injured. 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 an individual.

All 81 unclaimed deceased samples have generated DNA profile successfully. The sternum samples proved to be the best sample in this case. 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 while 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 acts as an ideal sample in mass disasters even exposed to 10% 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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