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Authors' Affiliation:
1- Forensic Services Laboratory, Centre for Applied Molecular Biology, University of the Punjab Lahore - Pakistan
2- Forensic DNA Typing Laboratory, National Centre of Excellence in Molecular Biology, University of the Punjab Lahore, Pakistan

***Corresponding Author:**
Muhammad Shafique
Email:
shafique@cemb.edu.pk

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Successful DNA Profiling for Identification of burnt Families from their bones using AmpFℓSTR Identifiler® Plus Kit

Muhamamd Shahzad[1], Muhammad Shafique[2*], Manzoor Hussain[1], Muhammad Adnan Shan[1], Rukhsana Perveen[1], Ziaurehman[2], Muhammad Idrees[1]

Abstract

Background: DNA profiling plays a vital role in the identification of dead bodies during mass disasters. Severe fragmentation, decomposition, burning and intermixing of the remains can occur in the mass disasters. DNA analysis faces many challenges especially when the dead bodies are completely decomposed or burnt. This report presents the identification of 32 completely burnt individuals including three families from their remains in a bus using AmpFlSTR Identifiler Plus® Kit and AmpFlSTR Y-filer® Kit.

Methods: DNA was extracted from provided remains of burnt bodies and reference samples by organic extraction procedure. The extracted quantity of DNA was calculated on ABI SDS7500 real time PCR with Quantifiler® Human DNA Quantification Kit (Applied Biosystems). DNA samples of 32 completely burnt individuals including three families were amplified using AmpFlSTR Identifiler Plus® Kit and AmpFlSTR Y-filer® Kit. The genotyping of these amplified samples was performed on ABI 3130xl Genetic Analyzer.

Results: The resulting data obtained from Genetic Analyzer was analyzed using GeneMapper ID software version 3.2 (Applied Biosystems). Seventeen burnt individuals including 3 burnt families were identified with the help of 16 autosomal STRs and 6 were identified through Y-STR analysis by allele sharing of their provided reference samples of parents and brothers respectively.

Conclusion: For the identification of unknown individuals particularly burnt deceased victims, STR analysis has become the gold standard in forensic science. Successful DNA profiling through the amplification of STR markers of AmpFlSTR Identifiler Plus® Kit proved to be very helpful in identifying the remains of burnt individuals even in the presence of inhibition observed in the Real Time PCR.

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Introduction

A disaster is an abrupt and unanticipated occurrence which results in causalities or injuring many victims. It may occur due to road accidents, natural disasters, human errors, technical evils (fires, explosions), terrorist attacks and even wars [1]. During sudden fire, skin of the burnt victims is lost and muscle tissues being exposed are ruptured. Burning of the face and skull makes the visual and dental identification of victims impossible. Burning of hands and feet makes the conventional fingerprinting impossible [2, 3]. Then, DNA profiling is recognized as one of the key modes of identification as part of the Disaster Victim Identification (DVI) response [4]. As per recommendations of International Society for Forensic Genetics (ISFG), twelve autosomal Short Tandem Repeat (STR) loci are recommended as the standard set for DVI; however sixteen or more loci are preferred [5].

In this report a passenger bus got unrestrained by the driver and hit the backside of an oil tanker at Super highway near Noriabad, Hyderabad, Pakistan. Both the vehicles instantly caught fire by the spreading out of petroleum. More than 50 passengers including men, women and children were travelling in the bus. At least 32 people lost their lives by complete burning in the fire and remaining faced to injuries. DNA analysis was focused to identify the families and individuals from remains of 32 burnt bodies.

Methods

Remains of 32 burnt bodies were received including teeth, jaw bones, femur bones, humerus bones, radius bones, rib bones and finger tips. Blood samples of 24 relatives/claimers were received as reference samples. DNA was isolated from all remains of burnt bodies and reference samples using organic extraction procedure. Prior to extraction teeth and bones were cleaned by running tap water followed by 100% ethanol to remove the debris and any microbial growth. The surface of the selected bones was cleaned using dermal tool. Teeth and bones were powdered by using the pre-sterilized filers. DNA quantification was performed by ABI SDS7500 real time PCR system using Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Catalog no. 4343895) according to manufacturer's protocol. Amplification was performed on GeneAmp PCR system 9700 using AmpFlSTR Identifiler Plus® Kit (Catalog no. 4427485) and AmpFlSTR Y-filer® Kit (Applied Biosystems, Catalog no. 4385124). Amplified PCR products were run on ABI3130xl Genetic Analyzer and data was analyzed using GeneMapper ID software version 3.2 (Applied Biosystems).

Results

The genotype data was analyzed and it was observed that all STR loci were amplified successfully from each DNA sample of burnt remains (Table 1). Among 32 burnt deceased victims, 17 were identified through autosomal STRs along with three burnt families and 6 were identified through Y-STR analysis by allele sharing with the DNA profile obtained from their reference samples of parents and brothers respectively. However, DNA profiles obtained from 9 individual burnt remains were left unknown due to the lack of their reference samples. In addition to this, pedigrees of three burnt families were also drawn as their members were identified (figure 1).

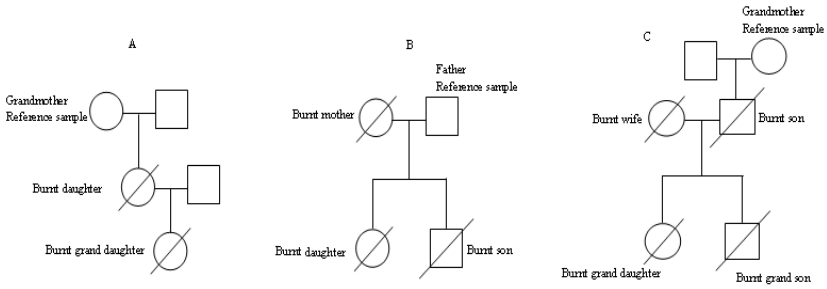

Figure 1: In family 'A' burnt daughter and burnt granddaughter were identified. In family 'B' burnt mother, her burnt daughter and her burnt son identified. In family 'C' burnt son, his burnt wife and their burnt siblings were identified. Individuals remained unmarked in the pedigree as their samples were not available.

In the 1st family, a burnt daughter and burnt granddaughter were identified through allele sharing at each locus with reference sample of grandmother (Fig.1A). In the 2nd family, DNA profile obtained from burnt son and burnt daughter shared one allele at each locus with the DNA profile obtained from their burnt mother and reference sample of their father (Fig.1B). In the 3rd family, DNA profile obtained from a burnt son shared one allele at each locus with the DNA profile obtained from the reference sample of his mother and further DNA profile obtained from his burnt wife found shared one allele at each locus with the DNA profile of their burnt children (Fig.1C).

Probabilities of paternity and likelihood ratios for burnt family members were also calculated as given in the table 2. The LR (likelihood ratios) values showed that

Sex	n	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539
Female	10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Male	22	22/22	22/22	22/22	22/22	22/22	22/22	22/22	22/22
Sex	n	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
Female	10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Male	22	22/22	22/22	22/22	22/22	22/22	22/22	22/22	22/22

Table 1: Identifiler Plus Markers Typeability of burnt victims DNA profiles.

		Probability of Paternity %	Likelihood Ratio
Family A	Burnt Daughter	99.9673	18.6141
	Burnt Mother	99.9680	14.1557
	Grand Mother (Reference)	-	-
Family B	Burnt Daughter	99.999999	403.540
	Burnt Son	99.999994	242.060
	Burnt Mother	-	-
	Father (Reference)	-	-
Family C	Burnt Daughter	99.999979	135.220
	Burnt Son	99.999994	185.190
	Burnt Mother	-	-
	Burnt Father	99.9955	27.750
	Grand Mother (Reference)	-	-

Table 2: Probabilities of Paternity and likelihood ratios for the burnt family members

the tested individual was more likely to be the father or mother of the child as compared to the other closely related individual of the child. Paternal lineage of six burnt male individuals were determined and identified as family members with reference to their brother samples through DNA profiling of 17 Y-STR markers.

Discussion

In this report successful DNA profiles were generated from 32 burnt remains. PCR inhibition was also observed in the results obtained from real time PCR in 21 samples. The DNA samples which were inhibited partially during Real Time PCR that were diluted up to the ratio of 1:100. The samples resulting with complete inhibition were treated by Thiopropyl-Sepharose cleaning (SIGMA-ALDRICH, Catalog no. 086K1943). Then 0.5 µL from each of these samples was used for amplification. The complete DNA profiles were obtained and no any drop out of alleles or loci observed in such samples. The use of Identifiler Plus® Kit for the amplification of burnt deceased victim's specimen demonstrated 100% efficiency as compared to Identifiler Kit [6] and Restriction Fragment Length Polymorphism (RFLP) Technique [7] that were used for the identification of burnt human remains.

A high success rate of getting DNA profiles was achieved using Identifiler plus markers in the first attempt from all the burnt remains which was absolutely challenging for individual identification. However, proper cleaning of the body remains to remove external DNA/contaminants/inhibitors must be ensured. Interpretation of STR data should be ensured for authenticity of results and inter-comparison of all the DNA profiles should be done to identify families in the mass disasters.

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