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Case Report

Disaster Victim Identification by DNA analysis: The Tazreen Fashions Garment Fire Incident Experience in Bangladesh

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ABSTRACT: DNA-based identification is considered one of the most important tools in the event of mass fatality incidents. This technology not only provides the most powerful method for identification, but also reduces the risk of misidentification by visual methods or by means of associated documents found with the dead body. The use of biometric methods like, fingerprints, radiology or dental records are also limited in mass disaster situations involving mutilation of dead bodies. DNA-based identification therefore, remains the most reliable method for the identification of victims, where the dead bodies are highly decomposed, severely burnt or disintegrated into parts. DNA bases identification involves the comparison of DNA profiles of postmortem samples with those of antemortem DNA samples (personalized items e.g. shaving razor, tooth brush etc.) or by kinship analysis with living biological relatives. The identity of the missing person is finally evaluated by likelihood ratio (LR) calculations after DNA profile comparison. In this study, we report the DNA identification effort of victims in the tragic fire disaster at Tazreen Fashions Ltd., a garments factory located in Savar, Dhaka. Out of 59 unidentified dead bodies, the identity of 43 was confirmed by DNA analysis with the participation of 68 biological relatives representing 61 families.

KEYWORDS: mass disaster, missing person, DNA-based identification, Short Tandem Repeats.

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INTRODUCTION

On November 24, 2012, Bangladesh experienced one of its worst fire disasters in the Tazreens Fashions factory, a garment factory located in the Ashulia District on the outskirts of Dhaka. The fire started on the ground floor of the nine storied building and quickly spread to other floors. It trapped most of the factory workers much like what happened in Triangle Shirtwaist factory fire more than 100 years ago in Manhattan, New York. The devastation killed 112 factory workers leaving at least 200 injured.

Following a mass disaster, besides rescue operation and medical support to the survived victims, proper identification of the dead victims is also very important. It is not only essential for humanitarian and emotional reasons but also for civil or criminal investigative purpose. The identification effort may sometime represent daunting challenge to the disaster victim identification (DVI) team or forensic investigators as the level of complexity largely depends on the nature of the disaster and factors involved in specific mass disaster scenario. According to the INTERPOL protocol for DVI the primary and most reliable methods of identification are friction ridge analysis, comparative dental analysis and DNA analysis.[1] Associated documents such as, clothing, jewelry, pocket items, badges, mobile phones or ID cards found with the dead body falls under secondary means of identification. Careful medical inspection of the body such as, age, sex, congenital conditions, healed injury, tattoo, body piercing etc. may also help the identification process. However, these identification procedures largely depend on the integrity of the dead body and the reliability decreases with the extent of body fragmentation or decomposition. Photograph or visual identification by a witness may provide an indication identity but they are least reliable and should not be used as the sole means of identification.

As for the Tazreen fire victims, most of the dead bodies were burnt superficially. So, tissue samples collected from deep muscles or hard tissues like bone and tooth samples served as the major source of DNA for the identification process. In this study the identification effort of 59 burnt dead bodies recovered from the disaster site is reported here by DNA analysis.

MATERIALS AND METHODS

Samples for DNA analysis

Tissue samples (n = 35) were collected from the least burnt area from the dead bodies. Bone (n = 2) and tooth (n = 22) samples were collected from bodies that were severely burnt. About 2-3 mL of peripheral blood was collected from biological relatives in an EDTA tube. Both blood and tissue samples were stored at -20^{o}C until analyzed.

DNA extraction

DNA from tissue samples were extracted by Geneaid™ DNA Isolation Tissue Kit (Geneaid Biotech Ltd., New Taipei, Taiwan). DNA from bone and tooth samples were extracted by using modified silica based method as described by Hasan et al.[3] Chelex-100 method was used for the extraction of DNA samples from peripheral blood samples collected from living relatives as reference sample.[4] Extracted DNA was quantified by using NanoDrop-100 (NanoDrop Technologies, Inc., Wilmington, DE 19810, USA.

PCR amplification

Approximately 1-2 ng of template DNA was used for PCR amplification process. All the loci included in AmpF*l*STR Identifiler™ PCR amplification kit (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) were amplified in using a Veriti thermal cycler (Applied Biosystems, USA). Depending on the relationship, Y-chromosome STRs and X-chromosome STRs were analyzed using AmpFlSTR Yfiler (Applied Biosystems USA) and Argus X-12 (Qiagen, Germany) respectively. Thermal cycling parameters were set up according to the manufacturer's protocol.

Capillary electrophoresis and STR typing

The PCR products were separated by capillary electrophoresis on ABI Prism 3100 avant Genetic Analyzer (Applied Biosystems, USA) using POP-4 polymer and data collection software ver. 2.0. Peak sizing and genotyping assignments were done by GeneMapper ID ver. 3.2.

Kinship analysis

Kinship analysis was done by comparing autosomal STR DNA profiles of samples collected from the dead bodies with the DNA profiles obtained from the available nearest kin. The significance of DNA profiles match for specific biological relationship was evaluated by likelihood ratio (LR) calculations using GenoProof® 2 software (Qualitype GmbH, Germany).[5]

RESULTS AND DISCUSSION

In Tazreen Fashions garment factory fire accident, a total of 112 people were killed. Due to the chaotic nature of the event and inadequate coordination, sequential steps in standard disaster victim identification procedure could not be followed. Therefore, secondary means of identification as described in INTERPOL DVI Guide,[1] such as, clothing, jewelry, associated document, pocket items in addition to visual identification were used in the first place. Out of them 112 victims, a total of 59 dead bodies were brought to Dhaka Medical College for DNA analysis. The DVI team failed to identify these bodies after applying these modalities described above. These bodies experienced different degrees of superficial burn. Depending on the extent of deterioration 35 tissue samples, 22 tooth samples and 2 bone samples were collected from the dead bodies. Since antemortem samples were not available for testing, reference sample from 68 relatives representing 61 families were collected as reference sample.

Recovery of undegraded DNA remains the most challenging task in most of the mass disaster scenario. Fortunately, we were able to get complete DNA profiles from all the tissues, bones and teeth samples collected from the dead bodies. This was due to the fact that the dead bodies were not subjected to any burial processes and the samples were collected within 72 hours of death. After getting the DNA profiles from the reference sample, the next job was to compare the DNA profiles between the unknown and reference samples. Statistical inference was drawn based on a pair-wise comparison of the DNA profiles of the sample in question and a single/multiple family reference sample, and then ranking the likelihood ratios (LRs) for specific biological relationship.

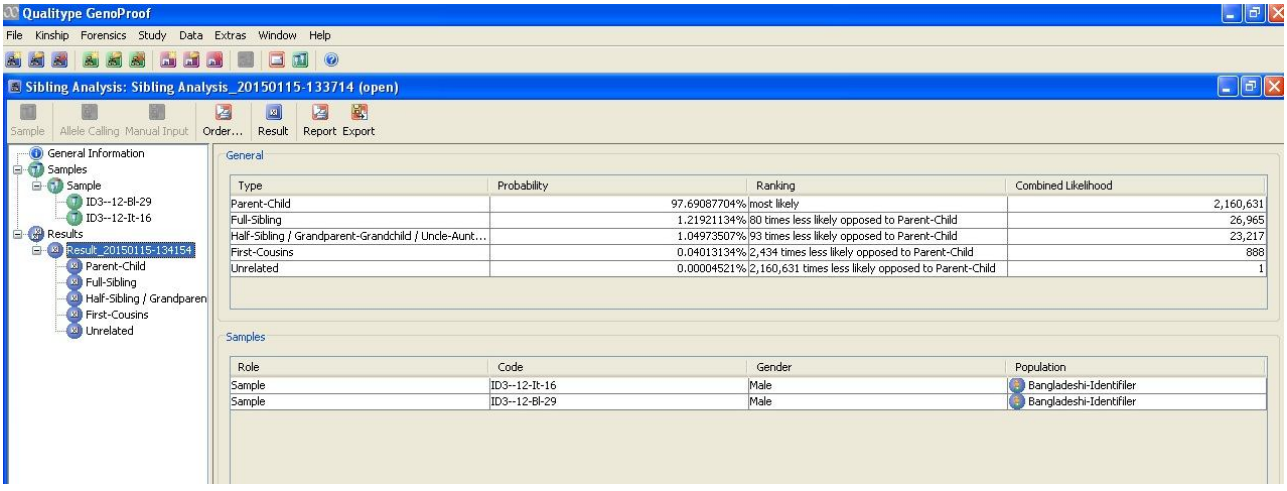

Figure 1. Genoproof output for likelihood ratio ranking.

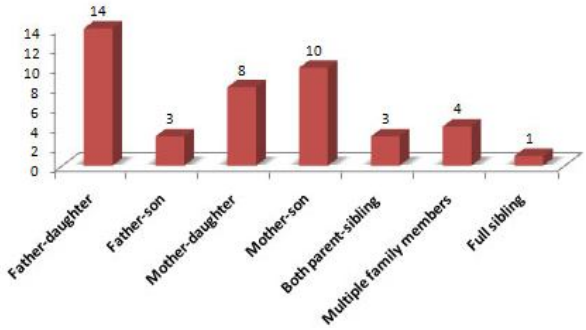

Figure 2. DNA match type of the identified victims.

The LR, which is also called the relationship index (RI) or kinship index (KI) is defined as; $KI = p_2\phi_2 + p_1\phi_1 + p_0\phi_0$ Where, ϕ_2, ϕ_1, and ϕ_0 are IBD (Identical by decent) coefficients. The IBD coefficients for major pairwise relationships were, the probabilities that two individuals might have 0, 1 or 2 alleles identical by decent [Parent-child: $\phi_2 = 0$, $\phi_1 = 1$, and $\phi_0 = 0$; Full-sibling: $\phi_2 = ¼$, $\phi_1 = ½$, and $\phi_0 = ¼$; Half-sibling: $\phi_2 = 0$, $\phi_1 = ½$, and $\phi_0 = ½$; First cousin: $\phi_2 = 0$, $\phi_1 = ¼$, and $\phi_0 = ¾$; Unrelated: $\phi_2 = 0$, $\phi_1 = 0$, and $\phi_0 = 1$;]. The LR depends on the probability of IBD allele sharing, the allele frequencies and the mutation model. We have used the allele frequency data from Bangladeshi population and an AABB (American Association of Blood Bank) mutation model supported by GenoProof® 2.[2] Each independent locus tested produces its own LR, which is then multiplied across to calculate a combined likelihood ratio for each pair-wise comparison at five different propositions, such as, parent-child, full-siblings, half-siblings, first cousins and unrelated. The GenoProof® software used here displayed the significance of match by ranking the calculated LRs for each five propositions with a threshold of 1.0 (Figure 1).

In the DNA identification effort of Tazreen Fashions fire victims, a combined LR for 15 autosomal STR loci was calculated for each possible pairs between the unknown victims and the family reference samples. The calculation was done by using GenoProof software v2.0 (Qualitype Germany). The result is presented in Table 1. The result shows that a total 43 unknown bodies were found to match with their family reference samples. Out of 43 matches there were 35 single parent-child matches (ID 1-3, 5, 8, 11-17, 19-21, 23-24, 27, 29-34, 36-41, 43-44, 48, 59-60) where either the child or parent was the victim. In all the cases both of them shared at least one allele with each other at all the 15 STR loci included in Identifiler™ kit. Out of these 35 matches there were 14 father-daughter, 3 father-son, 8 mother-daughter and 10 mother-son matches (Figure 2). Therefore, Y-chromosome and X-chromosome STRs were employed to further supplement the earlier matches found for autosomal STRs. Since Y-chromosome is a unique record of paternal inheritance a male child should share the Y-chromosome haplotypes with the father. So, all the father-son pairs were subjected to Y-chromosome STR analysis using Yfiler™ PCR amplification kit.[6] All the father-son pair shared the Y-STR profiles with each other, providing further evidence in favor of the autosomal STR match. Though other paternally related males (if any) would also share the same Y-STR haplotype with them, the tested relationship was confirmed as there were no other claimants.

As for the father-daughter, mother-daughter and mother-son pairs, X-chromosome STR analysis was used as a supplementary test using Agrus X-12 kit.[7] Ideally, a daughter should inherit the father's X-chromosome haplotype in its entirety. Similarly a son's X-chromosome haplotype should be reflected entirely in the mother's X-chromosome profile. In a mother-daughter situation on the other hand, a true mother-daughter should share at least one allele with each other at each locus much like autosomal STRs. In 31 cases out of 35 single parent-child match, the above three situations prevailed and X-chromosome STR match further confirmed the identity of the victims.

For ID-35, a brother was the reference sample donor for his brother victim. The sibling analysis yielded a very high combined LR (4,02,622) for full siblings. Their Y-STR match also indicated that they are likely to share a common father. In absence of other claimant the identity of the victim was confirmed.

In two cases there was a complete trio situation. For ID-09 both the parents donated reference sample for their daughter victim. For the other, a child donated reference sample for both of his parent victims (ID-28). In both the situations the victims were conclusively identified by standard parentage testing.

Multiple reference samples involving multiple victims were available for ID-04. Here, both the brother and sister donated reference sample for their mother and sister who were victim of the incident. In another case (ID-49) one girl donated reference sample for three victims of the same family, the father, mother and the brother. In both the situations autosomtal STR and X-chromosome STR analysis results fitted well in both the family pedigree.

This DNA identification effort of Tazreen Fashions fire accident could conclusively identify 43 burnt dead out of 59 brought under DNA analysis. The question remained, what was the identity of the 16 dead bodies that were left unidentified. A possible misidentification in the earlier identification methodologies may explain this. As mentioned earlier, a total of 112 factory workers were killed in this incident. About 50% of the dead bodies were initially identified either by visual methods or other identification modalities which did not follow any of the primary methods for identification described in INTERPOL protocol for DVI.[1] Any misidentification in the earlier stage might have reflected here. In effect, one misidentification lets one dead body in wrong hands, one dead body left unidentified and one family in agony. Therefore, further DNA analysis by including all the body/Body remains handed over without DNA test and the family members who did not participate in DNA analysis, could possibly provide a complete picture about the identity of the victims who are still left unidentified.

Table 1. Likelihood ratio ranking for pair-wise comparison of the DNA profiles.

Family ID	No. of Biological Relative	Reference Sample Donor	Missing Relatives	Combined Likelihood for Kinship Analysis					Interpretation
				Parent-child	Full sibling	Half-sibling	First cousins	Unrelated	
01	Relative-1	Father	Daughter	1,11,361	439	1,595	79	1	Match found with victim-13
02	Relative-1	Son	Mother	4,537	1662	307	39	1	Match found with victim-35
03	Relative-1	Daughter	Mother	1,06,401	14920	2237	124	1	Match found with victim-30
04	Relative-1	Brother	Sister	0	23,861	1,147	102	1	Match found with victim-59
	Relative-2	Daughter	Mother	20,14,771	55,489	25,321	980	1	
05	Relative-1	Son	Mother	3,16,918	14,194	3,430	136	1	Match found with victim-33
06	Relative-1	Mother	Daughter	-	-	-	-	-	No match found
	Relative-2	Father	Daughter	-	-	-	-	-	No match found
07	Relative-1	Son	Mother	-	-	-	-	-	No match found
08	Relative-1	Father	Daughter	93,456	1,274	1,264	64	1	Match found with victim-19
09	Relative-1	Father	Daughter	3,66,440	2,13,616	2,756	128	1	Match found with victim-05
	Relative-2	Mother	Daughter	40,210	893	778	49	1	
10	Relative-1	Brother	Sister	-	-	-	-	-	No match found
	Relative-2	Mother	Daughter	-	-	-	-	-	No match found
11	Relative-1	Mother	Daughter	1,612	85	12	3	1	Match found with victim-52
12	Relative-1	Father	Daughter	1,43,791	1,986	812	97	1	Match found with victim-34
13	Relative-1	Mother	Daughter	1,50,252	2,746	536	136	1	Match found with victim-51
14	Relative-1	Father	Daughter	1,62,274	35,817	1,866	84	1	Match found with victim-43
15	Relative-1	Son	Mother	1,10,113	29,949	2,954	166	1	Match found with victim-47
16	Relative-1	Son	Mother	3,915	84	50	2	0	Match found with victim-11
17	Relative-1	Daughter	Mother	26,374	512	261	36	1	Match found with victim-22
18	Relative-1	Father	Daughter	-	-	-	-	-	No match found
19	Relative-1	Father	Daughter	27,039	619	550	38	1	Match found with victim-01
20	Relative-1	Father	Son	9,749	288	118	27	1	Match found with victim-31
21	Relative-1	Father	Son	1,581	94	62	14	1	Match found with victim-56
22	Relative-1	Brother	Brother	-	-	-	-	-	No match found
23	Relative-1	Son	Mother	26,637	685	227	52	1	Match found with victim-55
24	Relative-1	Father	Daughter	1,01,169	1,346	869	68	1	Match found with victim-28
25	Relative-1	Son	Mother	-	-	-			No match found
26	Relative-1	Mother	Daughter	-	-	-	-	-	No match found
27	Relative-1	Son	Father	5,93,836	14,707	8,213	326	1	Match found with victim-24
28	Relative-1	Son	Father	21,60,631	26,965	23,217	888	1	Match found with victim-16
			Mother	10,640	490	122	50	1	Match found with victim-29
29	Relative-1	Son	Mother	1,86036	12,648	2,364	102	1	Match found with victim-09
30	Relative-1	Daughter	Father	2,490	135	18	5	1	Match found with victim-17
31	Relative-1	Father	Daughter	11,47,885	1,03,077	14,111	461	1	Match found with victim-39
32	Relative-1	Mother	Daughter	10,976	430	422	40	1	Match found with victim-15
33	Relative-1	Son	Mother	16,99,018	29,966	11,300	303	1	Match found with victim-26
34	Relative-1	Father	Daughter	6,05,51,094	66,60,193	2,20,642	2,825	1	Match found with victim-08
35	Relative-1	Brother	Brother	4,02,622	6,22,218	6,564	233	1	Match found with victim-04
36	Relative-1	Mother	Daughter	16,32,301	11,661	8,351	314	1	Match found with victim-27
37	Relative-1	Father	Daughter	1,65,869	16,708	3,033	154	1	Match found with victim-02
38	Relative-1	Daughter	Mother	7,232	3,127	322	33	1	Match found with victim-50
39	Relative-1	Son	Mother	83,823	2,265	872	149	1	Match found with victim-46
40	Relative-1	Son	Mother	17,55,338	3,31,834	19,248	683	1	Match found with victim-21
41	Relative-1	Mother	Daughter	1,62,133	3,451	2,008	91	1	Match found with victim-54
42	Relative-1	Father	Daughter	-	-	-	-	-	No match found
43	Relative-1	Son	Mother	44,84,585	27,009	26,116	654	1	Match found with victim-48
44	Relative-1	Father	Daughter	5,049	1,656	244	26	1	Match found with victim-45
45	Relative-1	Mother	Daughter	-	-	-	-	-	No match found
46	Relative-1	Son	Father	-	-	-	-	-	No match found
47	Relative-1	Son	Father	-	-	-	-	-	No match found

Table 1. Continued.

Family ID	No. of Biological Relative	Reference Sample Donor	Missing Relatives	Combined Likelihood for Kinship Analysis					Interpretation
				Parent-child	Full sibling	Half-sibling	First cousins	Unrelated	
48	Relative-1	Father	Daughter	5,466	259	135	28	1	Match found with victim-14
		Daughter	Father	4,64,35,630	8,87,663	2,41,181	4,118	1	Match found with victim-36
49	Relative-1	Daughter	Mother	1,854	126	53	18	1	Match found with victim-25
		Sister	Brother	0	11,870	5,414	708	1	Match found with victim-12
50	Relative-1	Mother	Daughter	-	-	-	-	-	No match found
51	Relative-1	Brother	Sister	-	-	-	-	-	No match found
52	Relative-1	Brother	Sister	-	-	-	-	-	No match found
53	Relative-1	Son	Mother	-	-	-	-	-	No match found
54	Relative-1	Brother	Sister	-	-	-	-	-	No match found
55	Relative-1	Daughter	Father	-	-	-	-	-	No match found
	Relative-1	Wife	Husband	-	-	-	-	-	No match found
56	Relative-1	Mother	Daughter	-	-	-	-	-	No match found
	Relative-2	Sister	Sister	-	-	-	-	-	No match found
57	Relative-1	Uncle	Nephew	-	-	-	-	-	No match found
	Relative-2	Grandmother	Granddaughter	-	-	-	-	-	No match found
58	Relative-1	Brother	Sister	-	-	-	-	-	No match found
59	Relative-1	Father	Daughter	1,86,639	4,131	2,183	254	1	Match found with victim-58
60	Relative-1	Father	Daughter	45,129	1,700	238	132	1	Match found with victim-40
61	Relative-1	Mother	Daughter	-	-	-	-	-	No match found

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