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Comparison of DNA Yield and STR Profiles from Different Tissue Samples for Identification of Corpse in Mass Disasters

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ABSTRACT

Introduction: In mass disasters, the extent of body fragmentation challenges the disaster response planning especially the identification process. The proper sample selection reduces stress for those involved in the identification process thus increasing the probability that all recovered samples are identified.

Aim of the work: To determine the preferred human tissue for DNA fingerprint for human identification for available samples in an airplane crash.

Materials & methods: In an air crash, 229 samples from different tissues were collected. DNA extraction was done using Qiagen automated kit by EZ1 DNA Investigator. Extracted DNA was then quantified using Quatifiler® Human DNA Quantification kit by Step one Real Time PCR. Samples were amplified using AmpFlSTR ® Identifier ® Plus PCR and AmpFlSTR ® Y filer Amplification Kits. Amplified PCR products were run electrophorethically on a 3130xl or 3500 Genetic Analyzer and finally analyzed.

Results: Comparison between various tissues recovered showed that dry blood stain have better DNA yield and STR results followed by fresh tissue then hair samples containing roots. Moreover, cartilage samples were preferred to bone samples concerning DNA yield and STR results. Finally, skin tissue then teeth samples had the least DNA yield and STR results.

Conclusion: Dry blood sample, if available, is the best sample type for DNA fingerprint in mass disasters. If not available, fresh muscle tissue then hair with roots can be used. Cartilage gives better results than bone concerning DNA yield and STR results leaving skin then teeth as the last choice.

Keywords: Disasters, Tissue samples, Human identification, DNA yield STR profiles

INTRODUCTION

Modern DNA typing technology has used new methods to perform human identity testing. This is of great importance in a number of situations including the determination of assailants of violent crimes such as murder and sexual assault, and identification of remains of missing persons or victims of mass disasters [1].

Disaster victim identification (DVI) for mass fatality incidents involving highly fragmented or degraded remains is a difficult process because the individual's information that is required by the various identification disciplines is

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Besides advancing the investigative process, identification addresses critical human needs that constitute the most basic of all human rights for both the victim and the family members [3].

Proper identification allows notification to the family of the victim and identification of their missing loved one, which may lessen the emotional stress by resolving uncertainty about the missing and promoting earlier reunification with the remains [4].

Identification is also an essential requirement for issuing an official death certificate that allows relatives to claim insurance benefits, access family assets and receive property that is in the name of the missing person. Therefore, failure to promptly and accurately identify human remains may have severe emotional and financial implications for the family of the missing and presumed deceased [5].

DNA regions with repeat units that are 2-6 bp in length are called microsatellites or short tandem repeats (STRs) [6]. The number of repeats in STR markers is highly variable among individuals, making these markers effective for use in forensic applications [7,8].

Polymorphic STR loci can be analyzed simultaneously by multiplex PCR. The Federal Bureau of Investigation (FBI) uses a standard set of 13 specific STR regions for CODIS [9].

Also, the analysis of short tandem repeat (STR) markers located on the Y chromosome is an important method in forensic casework analysis. Y-STR analysis is able to detect the presence of minute amounts of male DNA of one or multiple donors and resulting genetic profiles can be compared to known reference samples [10].

The current study highlights the importance of proper sampling and the right choice of tissues for DNA identification that will surely augment the probability of identification of nearly all tissue fragments and subsequently identification of almost all victims.

THE ACCIDENT

The accident was an airplane crash over the Mediterranean of Egypt air flight 804 in May 2016. All the passengers drowned and most of the victims' bodies were fragmented. Efforts for recovery of victims and body parts continued for nearly a month. The airplane crash cause was not precisely identified but hypothesis was claimed by investigators suggesting environmental factors e.g. bad weather. On the other hand, others suggested a terroristic bomb explosion or plane system failure as component malfunction.

SAMPLING

About 229 samples from different tissues **(Table 1)** were recovered from the crash and sent to DNA laboratory in at the Egyptian Forensic Medicine Authority (EFMA) at Sayda Zaynab for preparation and DNA extraction.

Precautions to avoid contamination during handling and shipping of the samples from the site to the DNA laboratories are also recommended. All samples need to be individually wrapped in packing material [11].

Table 1. Type of tissue examined in aircraft crash disaster.

Tissue type	Count
Cartilage	94
Bone	98
Dry blood stain	2
Skin	10
Tissue	16
Hair	7
Teeth	2

Reference samples from relatives of victims were also collected (Buccal swabs and fresh blood samples). The samples were labeled with their names and the victim's code or name.

Dried blood samples preparation

Dried blood specimens were obtained from different parts of the corpse that contain blood. Blood was spotted to a sterile gauze approximately (2 cm×2 cm) diameter then allowed to dry at room temperature or at 37°C.

In cases of scarce blood, a foam-tipped buccal swab was inserted into an incision, which was held open by disposable tweezers, and pressed or "rubbed" into the muscle or tissue containing blood until saturated. The swab is left to dry to be processed later.

Tissue samples preparation

For tissue samples that ranged from normal (red and soft) to burnt (dark brown and brittle) recovered from muscle or tendon tissue, we cut about 1 g of the tissue using a sterile blade, minced it well using a sterile blade to start operating on.

Hair samples preparation

For hair samples approximately 1-5 plucked hair fibers either plucked manually as scalp hair or with forceps were used for extraction. Nearly 1 cm of hair along with the root was plucked and placed in a sterile 1.5 ml tube, identified, labelled. The samples were washed with sterile deionized water then vortexed. The supernatant was discarded. The wash was repeated three times**.**

Bone and teeth sample preparation

Bone sample was de-fleshed, cut to (2 cm×2 cm) and scrubbed with a sterile brush and washed with distilled water. Samples were then decontaminated with a series of 20 % bleach, distilled H_2O, and 70% ethanol three times then dried.

For teeth, multi-rooted teeth or canines were harvested as they have the largest DNA-containing pulp cavities.

Bone and teeth samples were dried in the oven at 37°C for 18-24 h. After drying, Samples were pulverized into fine powder by means of a scalpel.

150-200 mg of powdered bone was placed into a 2 ml micro centrifuge tube in which the bone powder is incubated with 750 µL of (EDTA) at (PH) 8.3 at 37°C in a thermal shaker for 2 days.

Cartilage samples preparation

For cartilage samples, about 500 mg of cartilage was cut by a sterile blade to small parts or chips or even crushed then treated the same way as pulverized bone powder by addition of 750 µL (EDTA) and incubation for at 37°C in a thermal shaker for about 2 days.

DNA ANALYSIS

DNA extraction

DNA extraction from all samples was done using Automated Qiagen extraction by EZ1 DNA Investigator (Qiagen, Germany) according to the manufacturer protocol.

DNA quantitation

All DNA quantifications were performed using the human DNA quantification kit Quantifiler TM (Applied Biosystems, USA*)* on a Step one Real-Time PCR system.

DNA amplification and STR analysis

It was done using AmpFlSTR Identifiler PCR amplification **kit (Applied Biosystems, USA)** and for Y STR The AmpFlSTR® Yfiler™ PCR **(Applied Biosystems, USA)**. Amplicons were separated using a genetic analyzer **(ABI 3130x; Applied Biosystems, USA)** and analyzed with the appropriate software **(Gene Mapper *ID*, version 3.2; Applied Biosystems)** using standard procedures**.**

RESULTS

By DNA quantitation, the study showed that dry blood stain samples gave the highest results of DNA yield among tissues examined in aircraft crash disaster whereas teeth and skin gave lowest results of DNA yield **(Table 2).**

Table 2 also shows the relation between numbers of samples delivered to their DNA yield revealing that despite dry blood stain samples show the lowermost count delivered from the aircraft crash disaster, it gave the highest maximum DNA yield 169.00 ng/100 mg/µl In the other hand, bone that shows the highest count of delivered samples from the aircraft crash, it gave a maximum DNA yield of only 4.01 ng/100 mg/µl.

Table 2. DNA yield from various types of tissues examined in aircraft crash disaster.

	DNA yield (ng/100 mg/µl)							
Tissue type	Count	Minimum	Maximum	Mean	SD	25th percentile	Median	**75th percentile**
Cartilage	94	0.00	15.90	1.59	2.46	0.18	0.70	**2.00**
Bone	98	0.00	4.01	0.47	0.77	0.06	0.16	**0.43**
Dry blood stain	2	38.31	169.00	103.66	92.41	38.31	103.66	**169.00**

Skin	10	0.00	0.04	0.02	0.01	0.02	0.02	**0.04**
Tissue	16	0.08	113.65	32.17	28.47	12.81	25.87	**44.00**
Hair	7	0.00	49.25	14.75	16.77	0.47	14.27	**17.33**
Tooth	2	0.00	0.01	0.01	0.01	0.00	0.01	**0.01**

After STR analysis, we succeeded to get a full STR profile from about 198 collected samples from the aircraft crash disaster with 86.5% success rate **(Table 3)**.

Table 3. Profile identification in aircraft crash disaster (STR success rate).

Profile	Count	Column N %
No profile	31	13.5%
Full profile	198	86.5%

By comparison of STR profiles from different tissues, we found that all the dry blood stain samples and tissue samples gave full STR profile followed by cartilage (97.9%), hair containing root (85.7%), bone (81.6%) then skin (20.0%) while teeth samples gave no STR profile **(Table 4)**.

Table 4. Comparison of profile identification from various types of tissues examined in aircraft crash disaster (STR success rate).

	Profile			
	No profile		Full profile	
Tissue type	Count	Row N %	Count	Row N %
Cartilage	2	2.1%	92	97.9%
Bone	18	18.4%	80	81.6%
Dry blood stain	0	0.0%	2	100.0%
Skin	8	80.0%	2	20.0%
Tissue	0	0.0%	16	100.0%
Hair	1	14.3%	6	85.7%
Teeth	2	100.0%	0	0.0%

DISCUSSION

Recently, large scale disasters resulting in the fatality of tens to hundreds of thousands of people are increasingly prevalent [12].

The collection of a postmortem sample is more complicated. Complications are for instance: The highly variable degree of preservation of the human remains, and the high risk of cross contamination and challenging identification process [2].

The study aimed to compare the DNA yield and the resultant STR profiles of different tissues recovered an air crash accident to aid in maintaining a strategy of sample preference for DNA identification and avoid failure of DNA extraction thus saving time and money and showing respect to the victims' relatives through delivering the appropriate DNA results of identification as soon as possible.

According to this study, preferred sample type for DNA extraction and detection as revealed by the study was primarily blood dry stain samples that gave the highest DNA yield and a complete STR profile. It was followed by tissue samples, hair then cartilage and bone samples. The teeth gave least DNA yield and bad STR results.

This agrees with the study that was done by Wheeler et al. (2017) [13] who revealed that bone marrow samples gave in the highest DNA yields, the minimum DNA degradation, and greatest STR success. However, several muscle, hair, and nail samples generated higher STR success rates than conventionally collected bone and tooth samples.

Kaneko et al. [14] also agrees to our results as he revealed that the rib could be an alternative hard tissue sample for DNA analysis to teeth samples according to studying samples of various hard tissues presented by 37 teeth, 42 skull, 42 rib and 39 nails from 42 individuals.

Also, Weed and Baum [15] agrees with current study in that in case of fresh body, dried blood swabs is preferred for DNA analysis. They in addition stated that the ribs are preferred for moderately decomposed remains and long bones are preferred for older remains. On the other hand, they state that in case of extensively fragmented remains post-mortem samples should be taken from red muscle.

Despite this study revealed a success STR rate for muscle tissue of 100% in aircraft crash. Rerkamnuaychoke et al. [16] in his study revealed that the DNA profile of muscle showed only 50% detected STR alleles. The variations in them may be due to differences in tissue decomposition depending on different factors such as temperature, humidity and PH of each environment.

In the present study, hair roots had a success rate of 85.7% taking into consideration that these results are probably due to availability of hair roots in the delivered samples. Watherston et al. (2018) totally disagrees with that in his study and he stated that hair is associated with use as an ante mortem (AM) sample is not a good source of DNA for the purposes of human identification of a PM sample [17].

In contrary to our results that showed than cartilage have better DNA yield than bone, Ferreira et al. [18] concluded that there was no difference between the quantity of DNA recovered from cartilage and bone samples by comparing DNA yields and DNA profiles of 20 cartilage samples with 20 bone samples from phalanges collected from the same victims of the largest natural disaster in Brazil [18].

These results may be an indication that Ferreira samples weren't subjected to a high degree of degradation or putrefaction which affected our samples resulting in the variation in the results between bone and cartilage in our study.

Also, Calacal et al. [19] disagrees with our results which stated the least DNA yield from teeth as he reported that teeth have better success rate than clavicle, ulna and radius.

CONCLUSION

The presented study concluded that sample selection strategy sequence in disasters and fatality incidents according to the available tissues recovered should be in the following sequence: Dry blood samples (or swabs) are the first choice.

If dried blood is not available, switch to tissue samples mostly muscle tissue or tendons, then hair samples with roots. If the tissues mentioned above were not available switch to cartilage samples then bone samples. The least preferred tissue for DNA test in disasters is teeth samples.

Right choice of samples following the established steps supports the creation of an accurate identification of deceased individuals and save time effort and resources.

COMPLIANCE WITH ETHICAL STANDARDS

Ethical approval: This article does not contain any studies involving human participants or animals performed by the authors.

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