

THE BENEFIT OF APPLICATION OF "STR TECHNOLOGY" IN IDENTIFYING THE CHARRED BODY OF A PREGNANT MOTHER AND HER FETUS

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ABSTRACT

Background: Identification of totally or partially burnt human remains and putrefied bodies is considered a serious forensic issue. In buried bodies, humus from soil can interfere with the DNA extraction process or may inhibit downstream amplification of the extracted DNA. In the present study, a partially charred body was found lying on its back with missing of both upper and lower limbs except the left humerus and half of the left tibia and fibula. In this cases, conventional fingerprint is impossible as the foot and hands became totally burnet. Therefore DNA usage is the main clue of this casa for perfect identification of the victim or highly putrefied bodies by "autosomal STR analysis" which became a method of choice for the identification of human remains", Method: Using of STR technology help in identification of the dead fetus and solve this case and identification of the suspect and prove that he is the father

Result: The present case report describes the application of DNA technology in identifying totally fired body, samples were taken from the victim and suspect, the result was the body of a pregnant woman who was put on fire after being killed by her boyfriend.

KEYWORDS DNA, STR, locus, fire, burn, identification

CASE PRESENTATION

Identification is considered an important objective in Forensic Medicine whether in cases of mass disaster or individual crises (Ali *et al.*, 2016)

The story begins on one winter night by discovering a burnt human body by the residents in the backyard of three multi-storied residential blocks in the district of El Sharabia (Cairo city, Egypt). Upon arrival at the scene, a partially charred body was found lying on its back with missing of both upper and lower limbs except for the

left humerus and half of the left tibia and fibula (figure 1). The body was surrounded by the ashes of burnt papers. The gender and the identity of the corpse could not be established at the scene because of the partial charring of the entire musculature. After conducting some basic crime scene investigations and interrogations from the residents, the body was released by the police to be transferred to the morgue for autopsy and other postmortem investigations.

In the morgue, the corpse was identified as a partially charred body with missing upper and lower limbs except the left humerus and half of the left tibia and fibula possibly because of 6th-degree burns (fig. 1)



Figure 1: The burnt body (corpse).

The body was presented with a pugilistic attitude due to the shrinkage of muscles and tendons. Upon opening the body, the internal organs were found to be reduced in size. Three plain AP X-rays were taken to identify the gender and any other underlying cause of death. Anatomy of the skull and pelvis revealed that the body was of a burnt female in her late thirties. Autopsy further revealed the presence of a dislocation of the right arm of thyroid cartilage and also in the left arm of hyoid bone [Fig. 4] as well as the present of uterus with erythema on its outer surface which appeared between the burnt molten mucosa and there is a dead fetus within about of 3.4 cm in length (the third uterine months) [fig. 2 and 3].



Figure 2 : The uterus with dead fetus inside it.



Figure 3 : The dead fetus.



Figure 4 : Dislocation of the left big arm of hyoid bone , and of the right big arm of the tracheal cartilage.

Samples of tissue from uterus, and the fetus as well as part of the burnt bone which in a good condition for DNA analysis. A part of the burnt tissue sent to the chemical laboratory to find out the cause of the fire. The policeman sent the suspect to the medical lab to take blood sample to compare it with the fetus sample and

the burnt body to see whether the fetus of the victim is his own son or not.

MATERIALS AND MRTHODS

Bone preparation

All bones were cleared mechanically, dried, washed, put in NaOCl 10% for a few minutes and washed again, milled (6×90s cycles) in liquid nitrogen using a cryogenic mill (6850 Freezer Mill SpexCertiPrep, USA).

We prepare each bone separately by using disposable sterile toolset, the room was designed specially for this procedure.

Extraction of DNA

The Bone powder was left overnight to be digested, phenol–chloroform–isoamyl alcohol was used to extract DNA, then purified and concentrated on micro-column "UltracelYM 100, Microcon" to 15 µl, incubation of 500 mg of bone powder with 2.5% NaOCl for 4 hours. Washing The pellet with water and ethanol 95% and suspended again in absolute ethanol, put in a centrifuge and left to dry over the night. The dried material was sonicated in ethanol 95%, put in a vortex and settled. Then washing the precipitant with 2.5% NaOCl and water and decalcified in 0.5 M EDTA pH 8.0, with a few exchanges of EDTA solution then centrifuged. The powder of the bone that was calcified incubated Bone powder that was calcified was incubated with proteinase K, DTT, EDTA, NaCl and SDS for thirty min at temperature 60°C and then left the whole night at temperature 37°C. The extraction of DNA was done by purification of phenol– chloroform–isoamyl alcohol, and concentrated by ultrafiltration (Ultracel YM 100, Microcon) to 15 µl. Total demineralization technique (TD) was used in extraction of DNA. Incubation

of 500 mg of bone powder all the night at 56°C In the buffer "0.5 M EDTA, 1% lauryl sarcosyl) and 20 ul of 20 mg/ml proteinase K, and concentrated and purified by using (Centricon Plus-20, Centricon 30 centrifugal filter unit (Millipore)". Appropriate control and decontamination processes were followed during all steps of extraction.

DNA quality:

Evaluation of the degree of Regaining of DNA and Extraction of DNA using PCR (polymerase chain reaction) inhibitors by Real-Time PCR with "QuantifierHuman kit (Applied Biosystems, USA).

STR genotyping:

Amplification was done by using "Identifiler1 Plus, AmpF ' STR1 NGMTM (Applied Biosystems, USA)". 9700 GeneAmp cycler was used for Cycling, the following design was performed (Initial denaturation for 10 minutes at temperature 95°C followed by 28 cycles 1 minute at temperature 94°C, for 1 minute at temperature 72°C then finally the last extension for 45 minutes at temperature 60°C. The reactions began with volumes 25 with DNA 10 then we get final concentration (from 0.5ng to 1ng), capillary electrophoresis was performed using "Applied Biosystems 3130xl geneticanalyseris" and profiling analysis was done using "GeneMapper ID v3.2.1."

RESULTS

The cause of burnt body death is asphyxia. The cause of death of the fetus is due to the stop of blood-feeding as a result of death of his mother by asphyxia (throttling pressure on the neck of the mother). The causal material of burning is Kerosene. The fetus is the son of both the burnt female and the suspect. (Table NO: 1)

Table 1: The Identifier STR profiles of corpse (burnt body), suspect, and the fetus.

STR Loci	Burnt body	Suspect	Fetus
D3S1358	15,16	15,17	15,16
CSF1PO	10,11	10,13	10,13
D7S820	8,10	11,13	10,11
D21S11	27,24	28,30	24,28
D8S1179	13,14	14,15	13,15
D19S433	12,14	15,15.2	12,15.2
D2S1338	17,17	18,18	17,18
D16S539	9,11	11,12	11,12
D13S317	10,13	11,12	10,12
THO1	6,10	7,8	6,8
FGA	22,28	21,26	22,26
D5S818	10,14	12,13	12,14
D18S51	13,13	15,17	13,15
TPOX	8,12	8,11	8,8
VWA	14,19	16,18	14,16

Using of STR technology help in the identification of the dead fetus and solve this case and identification of the suspect and prove that he is the father

DISCUSSION

In this study there is shrinking, loss of both upper and lower limbs with the fixation of tissues in whole body that agree with (Tosoko, 2004) that stated that the burnet body shows broad spectrum morphological finding

for example range from minor, local, superficial burns of the skin to calcined skeletal remains without any soft tissue left and total incineration.

In our study, there is successful DNA amplification with the AmpF^{STR1} Identifiler1 Plus AmpF^{STR1} NGMTM PCR Amplification Kit which agree with the study of (Tucker *et al.*, 2011) that stated that "the usage of AmpF^{STR1} NGM TM with the ESS loci is tolerant more commonly with the inhibitors so that we can face the obstacles in case of processing the skeletal remains that are compromised. unlike the study (Edson *et al.*, 2005) that stated that the burned remains became brittle, blackened and friable so failure rate of typing DNA is expected.

Genetic profiles can be determined according to the examination of samples collected from unknown deceased of unknown identity which is a routine test must be done in forensic laboratory, time place and surrounding circumstanced determine which biological sample is suitable for DNA extraction, in some case the bone, teeth, and nails of the deceased bodies is the only source for DNA extraction such as complete skeletonization, charred bodies a major decay of the crops as hard tissue are resistant to autolysis, many factors affect the degree of postmortem decomposition and in the same time affect DNA extraction. Factors such as humidity, high or low temperature, light water or dry air affect DNA extraction (Dragana *et al.*, 2015):

In our study, we took a sample for the DNA analysis from the tissue of the uterus, and 2 burnt ribs which were in a good condition. Like the study of (Kapiska and Szczerkowska, 2004) that stated that the process of genetic identification depends on these types of specimens that containing a large

amount of degraded DNA that usually depends on the analysis of microsatellite loci – STRs. DNA isolation by silica and a commercial kit (Promega) gave a full STR genotype only in the case of the burned body (Kapiska and Szczerkowska, 2004). In present casework, using AmpF^{STR1} Identifiler1 Plus was the right decision for getting complete DNA profiles from the burned bones.

Schwark *et al.*, achieved better results from DNA amplification of burnt bones obtained from actual cases (used autosomal STRs 1identifiler kit) (Schwark *et al.*, 2011), Shahzadet *et al.*, find a complete DNA profiles for the identification of 32 completely burnt persons including 3 families from their remains in a vehicle by Amp F1 STR Identifiler Plus® Kit and Amp F1 STR Y-filer® Kit⁹.

CONCLUSION

Extraction of DNA is an essential step when we want to recover a highly degraded and highly damaged DNA obtained from skeletal remains, and for DNA profiling used for the identification process of a missed person. Our study, shows that simple DNA extraction technique modifications will highly improve the success rate of DNA typing, and provide conclusive, reliable profiles using different amplification kits even when working with difficult samples. Our results show that the "AmpF^{STR1} NGMTM Amplification Kit" is highly accurate in genotyping the samples with degraded DNA samples by using STR loci and increase sensitivity and tolerability of common to improve the profiling from a very low quantity material of DNA.

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المخلص العربي

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يعد الاستعراف على الجثث المتفحمة كليا او جزئيا او الاستعراف على الجثث المتعفن رميا من احد اهم المشاكل فى علم الطب الشرعى، فى حالات الجثث المدفونة تعوق الاتربة و الشوائب الموجودة فى التربة عملية استخراج الحامض النووى DNA وفى بعض الاحيان تعوق عملية تضاعف الحمض المستخرج، فى هذه القضية .. وجدت جثة متفحمة جزئيا ترقد على ظهرها والاطراف السفلية والعلوية مفقودة ما عدا بعض اجزاء الجهة اليسرى ،، فى هذه القضية يعد الاستعراف عن طريق باستخدام بصمات الاصابع مهمة مستحيلة نظرا لفقدان الاصابع ،، يعتبر الاستعراف عن طريق الحامض النووى (DNA) هى الطريقة الامثل فى هذه القضية، تم اخذ عينات من الام والطفل والمشتبه به وتم حل القضية .