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Quantification of DNA profiling from burnt remnants

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***Abstract*---**In crime scene investigation, various evidences are attained in distinct forms by forensic experts. These evidences are often recovered in contaminated form, decomposed or partial form especially skelton remnants. In homicidal cases, dead bodies are redeemed partial burnt/ completely. In such cases, identification of an individual from highly burnt remnants is established by DNA profile. It can't be neglected that hard tissues (bones and teeth) can bear extreme fire impact and often attained with some partial flesh pieces. Although, a considerable degree of degradation may occur in DNA retrieved from burnt bone pieces. This study is mainly focused upon the extraction and quantification of DNA profile generated from such remnants. Effect of fire on DNA and extreme heat on blood, blood in form of prime source of DNA are believed to be no longer traceable after exposure to a temperature of 1000°C. The outcome of this research will help researchers to quantify DNA profile, identify the deceased person/ dead bodies from such cases.

***Keywords*---**burnt bodies, quantitative analysis, DNA profiling, burnt dead bodies, extraction, RT-PCR, crime scene.

Introduction

In course of forensic investigation, some certain situations arise in which burnt skelton remnants are recovered from scene of occurrence to establish the identity of victim. Whenever, bones are burnt, several physical and chemical properties change dramatically and make it difficult to set the personal identification of victim. Physical changes take place in the burnt bones in form of distortion and disintegration which alter the morphological measures that are critical for anthropometric analysis of sex, race and stature estimation. As a result of combustion and pyrolysis, heat generated during the burning process causes chemical modification of bones, in addition to physical changes in bone structure. The degree of change increases as the temperature rises, and this includes DNA degradation, which makes forensic identification techniques more difficult to use.
Fire victims in car accidents, victims of mass disasters, and victims of house fires are among the many types of cases for which burnt bones are submitted to the forensic laboratory. Beyond accidental homicide cases, the victim's body has been incinerated and destroyed with an intention to obstruct the inquiry[1]. It is difficult to make meaningful anthropological observations because of the heat-induced fragmentation of burnt bones, which is followed by artificial crushing. Furthermore, DNA analysis of bones that have been substantially burned is considered to be extremely challenging[3,4]. The partial burnt remains are the main source for extracting the DNA that may prove the identity of a person. Sometimes, it is challenging to extract the DNA when the body is fully burnt and thus proving the identity of a person is a tedious work. DNA is usually taken from bones as they are rigid and one can get good quality of DNA from bones but it is quite challenging and time-consuming task[2]. In year 2019, Naresh Kumar and his colleagues conducted a study on 'Effect of fire on DNA and its profiling in homicide cases' and concluded that DNA has proved its value in the identification of unidentified dead bodies or from the burnt cases[5]. By extending their work, Naresh Kumar and his colleagues published research entitled on Qualitative analysis of DNA profile attained from partial dead bodies in 2021[10]. This work is derived mainly from their work to extract and quantify the DNA from burnt dead bodies. It can help researchers to determine, whether DNA profile can be attained from burnt human remnants.

Methodology

Sampling

In this present study, all the samples (25 samples) were received from crime scenes such as mass disasters, accidental burning cases and homicidal which may have been exposed to harsh conditions such as heat, arson/ explosion cases and hot water that break down the chemical structure of DNA. All the samples were taken from the cases submitted in forensic science laboratory. Although, distinct environmental exposures & allied factors play vital role in degradation of DNA by breaking its molecules into smaller pieces. However, it is suggested to implement the legitimate provisions during the collection and preservation of these samples[6]. In this study, all the samples were collected from two places including body part directly exposed to the heat/temperature and secondary were collect from the interior part of body/parts near to the bone. This was conducted

to analyze to determine disparity in the quality and quantity of DNA from direct heat exposed body remnants.

DNA Extraction

All the samples were submitted in FSL for the examination. The samples were preserved at 4^{o}C to reduce the extent of degradation. DNA was isolated from the samples by using DNA extraction techniques such as phenol-chloroform extraction, FTA card, Automate extraction, Chelex extraction etc. Phenol-Chloroform method is quite sensitive technique used for DNA extraction[7]. Automate extraction is considered best method which is used for the degraded samples or samples that has faced high temperature. Extraction and purification of DNA analysis is primarily performed. For extraction, rupturing of cells and nuclear membrane is crucial to release DNA in the solution. Buffer solution and Proteinase K solution are used to rupture the cells and nuclear membrane. Proteinase K helps in the enzymatic digestion of proteins and non-nucleic acid components of the cell. After performing extraction, DNA is amplified by using PCR technique and analyzed by quantification[8]. The DNA isolated from forensic biological evidences provide an information to yield identification of the source. Isolation of good quality of DNA is a prime requirement in all the molecular genetic analysis. Phenol-chloroform method is extensively used for the organic extraction from the specimen. High molecular weight DNA can be attained most efficiently with phenol extraction. In organic extraction, buffer, SDS and proteinase K are added, and mixture is incubated at 56^{o}C[9]. For the digestion of cell and nuclear membrane, Sodium Dodecyl Sulfate (SDS) and proteinase K are added to breakdown the proteins that protect the DNA from lysis1[7]. The removal of protein is done by addition of phenol, chloroform and isoamyl alcohol followed by vortex and centrifugation. Pellet is washed numerous times and dried at room temperature.

Quantitation

Quantification of the DNA has a substantial role in DNA amplification and STR profiling. Several methods have been established to quantify DNA from basic UV spectrometry, through gel-based techniques, to dye staining, blotting techniques, and DNA amplification methods (PCR). After the isolation of DNA from the samples, the amplification of DNA is done by using Quantifier® Duo Quantification kit (Applied Bio systems) with 7500 Real Time PCR machine[11]. At present, RT-PCR or q-PCR is most popularly used in laboratories as it is reliable and accurate. It is quite sensitive in detection of contaminated DNA and used to amplify the DNA even if small amount of DNA is extracted from the samples. In case of unknown samples, Identifiler STR kit plays an important role in the identification. The quantification of DNA by qPCR depends on the detection of amplified product at each cycle of the PCR[12]. Thermal cyclers are extensively used in the detection of the PCR product by computing the real-time fluorescence changes due to the production of amplicon. The quality & quantity of the isolated DNA can be estimated by using gel electrophoresis and UV spectroscopy. For measuring the concentration of extracted DNA, UV spectrophotometer is used at 260nm and 280nm. The ratio between the reading at 260 and 280 nm provides

an estimation of purity of DNA. OD280 is corresponding to protein content. The concentration of extracted DNA can be calculated from optical density at 260 nm.

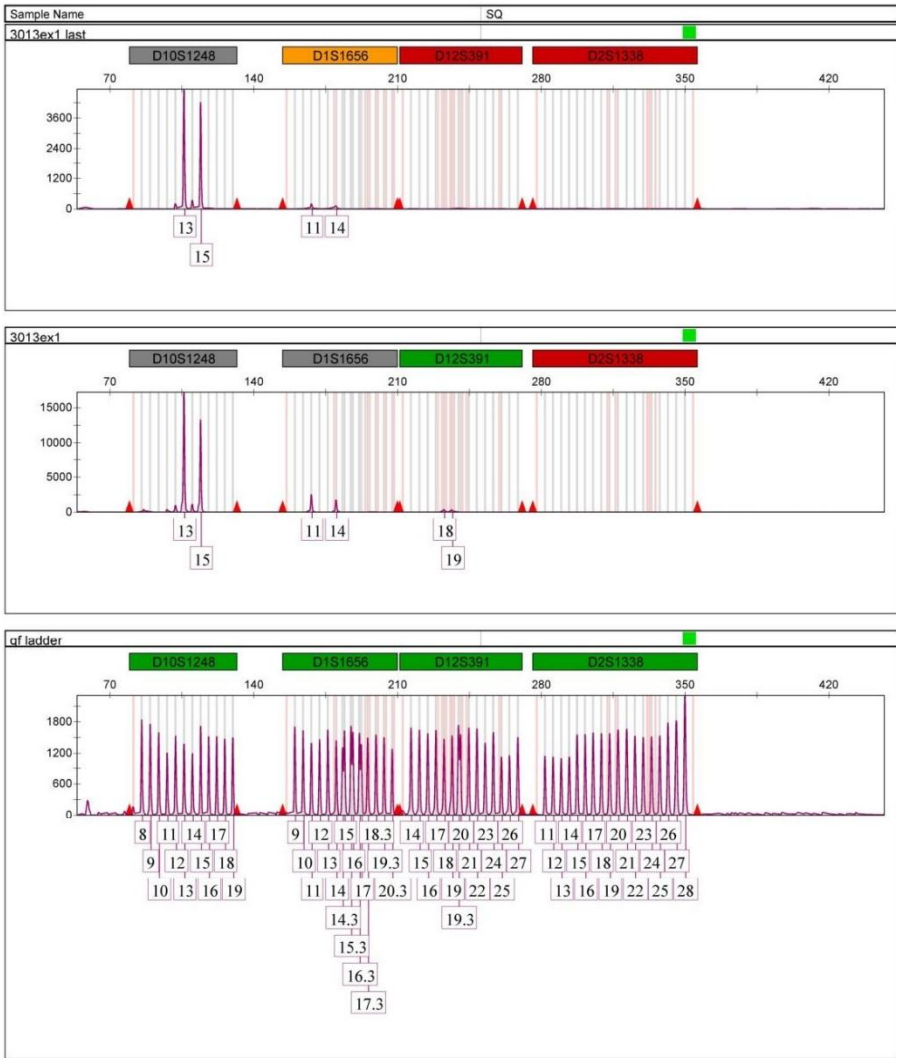
Result and Discussion

In burnt dead bodies, it is most challenging task for forensic experts to establish the identity of an individual. In these cases, forensic experts attain body tissues, bones/ teeth to generate DNA profiling and determine the identity of victim. The burnt tissues of muscles are recovered in dark brown/ blackish color and fibers are collected from the lower side of burnt material. In most of samples, when DNA was quantified; the attained quantity of DNA was very less/ negligible. During analysis, it was observed that Large autosomal target was most effected part which could not be amplified during RT PCR amplification[10]. The quantitative analysis of DNA of burnt bodies are given below in table no.1-

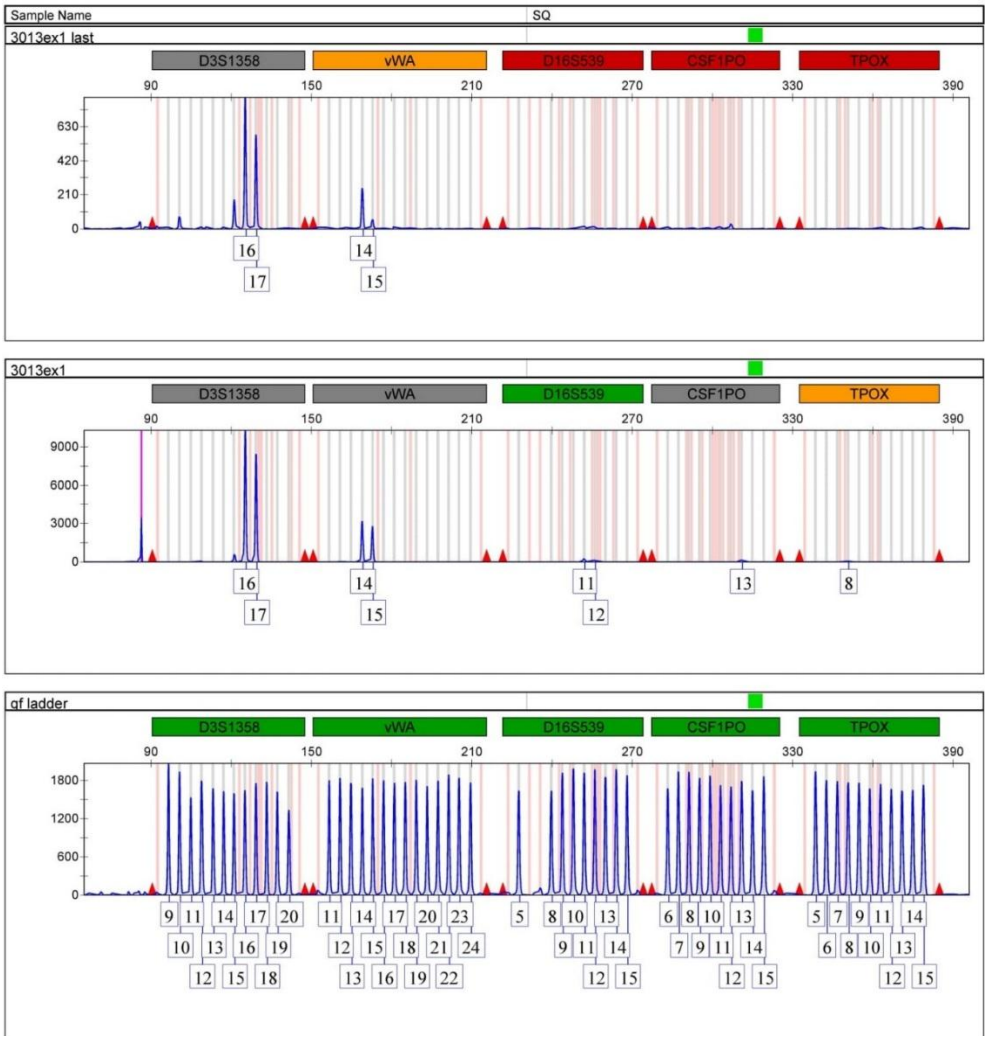
Table 1
Quantitative analysis of DNA from burnt bodies

Sample No.	T. Large autosomal (DNA in ηg./µl.)	T. small autosomal (DNA in ηg./µl.)	Y-(DNA in ηg./µl.)
1	-	10.76	9.76
2	-	13.25	2.76
3	-	6.23	5.97
4	-	13.21	3.38
5	-	8.17	8.13
6	0.00	00	00
7	.03	.06	.02
8	.03	.03	.12
9	.02	.02	4.68
10	-	-	-
11	-	8.79	3.68
12	-	4.56	5.91
13	-	0.21	0.35
14	-	5.98	6.13
15	-	7.12	6.14
16	-	0.23	4.65
17	0.04	0.16	7.89
18	0.01	1.03	12.03
19	-	-	-
20	-	11.79	9.36
21	.02	9.63	4.59
22	-	5.89	2.97
23	.03	10.09	3.83
24	0.1	3.86	8.16
25	-	-	-
Average	0.035	5.503	5.023

However, the small autosomal targets were less effected as that was not exposed to direct heat. Therefore, DNA was amplified from the small targets. As a resultant of this study, average quantity obtained from direct heat exposed bodies for large autosomal targets was 0.035 ηg./µl while average quantity of small targets was 5.5 03ηg./µl and Y DNA was 5.023 ηg./µl. The results of this study suggest that in burnt cases, partial DNA profile will be generated. It is also observed that if there is burnt tissues/ body parts have been found on the spot, the outer burnt portion should be removed from the surface and lower portion of muscles/ tissues should be taken for DNA profiling. The generated DNA profiles are given below in electropherogram no.1, and 2-



Electropherogram 1. Attained DNA profiling from the tissues of burnt remnants



Electropherogram 2. Attained DNA profiling from burnt tissues.

In these samples, STR profiles were analyzed that were devoid of PCR artefacts. Removal of inhibitor was observed effective in the isolation of high-quality genomic DNA. This work suggests that in burnt dead bodies, it is not necessary that all samples will generate Large autosomal DNA profile while some profiles have been given minimum amount of DNA. The presence of multiple peaks and imbalance of peak height was observed due the impact of fire[10]. It is very important to obtain appropriate quality & quantity of DNA to establish identity from the putrefied and unidentified burnt dead bodies. The study suggests that direct fire affected area of body tissue should not be taken for DNA analysis.

Conclusion

As a part of investigation, it is imperative that the identification of the victim should be established from any mean of evidence/remnants. The most of the time, offenders deliberately set fires in order to destroy evidence that could lead to

the identification of the suspect. DNA has proven its utility to determine the identity of unidentified deceased bodies as well as in the identification of burnt skelton remnants. It is an irrefutable fact that, DNA profile is accurate in establishing the identity of the victim. It is critical to acquire adequate quality and quantity of DNA for identification purposes from the bodies. Now a days, utilization of DNA to identify the deceased from the human remnants over an accidental/deliberately fire/arson/ mass disaster has become a standard technique in the scientific community[10]. This research paper will help researchers to quantify DNA profile, identify the deceased person/ dead bodies from such cases and to nab the suspect to put them behind the bars.

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