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Research Article

Quantitative Analysis Of Dna Attained From Partial Burnt Dead Bodies.

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ABSTRACT

In forensic investigation, dead bodies are recovered in legion conditions. Burning of corpses, frequently encountered in heinous crime scene cases such as suicide, homicide, accidents etc. The evidences attained from burnt remains are partially or fully destroyed. In case of partial burnt bodies, the burn extends to dermis. The blood gauze, partially burnt flesh pieces, burnt hair, nails etc. may be found in case of partially burnt dead bodies. The DNA can be isolated from the cells and tissues which were not in direct contact with fire. The partial burnt remains are the main source for extracting the DNA that may prove the identity of a person. In case of partial burnt remains, DNA can be obtained easily from partially burnt tissues and the quality of DNA is better in comparison to fully burnt remains. 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.

KEYWORDS. Quantitative analysis, DNA profiling, burnt dead bodies, extraction, RT-PCR, crime scene etc.

INTRODUCTION

Advancements in the field of forensic science has taken over the traditional methods in the investigation. Investigating officers encounter the crime scene and plays a vital role in the collection of evidences. Numerous types of physical evidences; such as fingerprints, blood, semen, saliva, fiber, hair etc. are frequently obtained from the crime scene. They may independently and objectively link a victim or suspect to the scene of crime, disprove an alibi, or develop important investigative leads. Investigating team visits the crime scene and collect all viable biological substantial's traces from the crime scene.

The biological fluids have a great importance in determining the type of crime. It has been observed that, the identity of a person can be revealed by a considerate analysis of DNA attained from biological samples. DNA evidences can be used to establish a link between person-person, person-other physical evidence, or person-crime scene. DNA analysis has become a crucial subject which aids in finding the personal identification in the field of forensic medicine and criminal science. Short tandem repeats (STRs) and Single nucleotide polymorphisms (SNPs) are some advanced tools that assist to scrutinize the extracted DNA from human remains, even from minute traces (less than 1ng) or highly degraded samples. Analysis of DNA is limited to only biological samples that contain nucleated cells. The biological evidences help to divulge the identity of the perpetrator in many crimes.

Burning of corpses, frequently encountered in heinous crime scene cases such as suicide, homicide, accidents etc. The evidences attained from burnt remains are partially or fully destroyed. In case of fully burnt dead bodies, burnt remains such as long charred bones, teeth are found from the crime scene. Due to burning, physical and chemical changes were seen in the properties of bones that might cause difficulties to discover the identity of a person. Physical changes have taken place in the burnt bone, such as distortion and disintegration which alter the morphological measures that are critical for anthropometric analysis of sex, race and stature estimation[1]. Degradation of DNA samples depends on the degree of heat (temperature) applied. A wide range of cases where investigating officer encounter partially and fully burnt dead bodies, including fire victims in vehicle accidents, from mass disasters, in house fires, self-inflicted burns etc[2]. In burning cases, as the information passes to the nearby people or to the police, they may try to control the fire by making use of water, sand, etc. (very common sources). In such circumstances, the blood patterns do not affect by the fire if the temperature is about 40^{o}C or less than of it[3]. The primary step of the investigating officer is to find the identification of a person and further analytical steps (if feasible) should be taken to clarify the mechanism of death[4].

Three type of burns are seen such as low intensity burns, medium intensity burns and high intensity burns. How much a body has been injured, depends on the intensity of the burn. The external as well as internal findings in the burned bodies depends on the actual temperature applied, time of burning, type of transmission of heat. Third degree burnt dead bodies are the high intensity burns in which body appears black, grey, brown, charred, or waxy[5]. These burns completely damage the skin. Partial burnt dead bodies are found very often from the crime scene[6]. They are recovered generally in case of self-inflicted burns, homicides etc. Petrol, diesel, kerosene is commonly used as a fuel in burn cases. This led to the cause of partial burnt dead bodies. In case of partial burnt bodies, the burn extends to dermis. The blood gauze, partially burnt flesh pieces, burnt hair, nails etc. may be found in case of partially burnt dead bodies[7]. The DNA can be isolated from the cells and tissues which were not in direct contact with fire. 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[8]. The DNA can be isolated from hair, nails, teeth, bones and from tissues which were not in direct contact with fire. Usually, DNA is extracted from scalp hair as if they are not in direct contact with fire[9]. However, hard connective tissues can protect the DNA for a long span even if the temperature exceeds than 100°C. In case of fully burnt dead bodies, 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[10]. While, in case of partial burnt remains DNA can be obtained easily from partially burnt tissues and the quality of DNA is better in comparison to fully burnt remains. The quantitation is quite easier in case of partially burnt dead bodies than the fully burnt dead bodies. Examine the difference in the quality of DNA when taken from burnt tissues (flesh) instead from other burnt remains (such as hairs, nail clippings, bones, teeth etc.)[11].

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. 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[12].

METHODOLOGY

Sample collection

During this study, all the samples collected from crime scenes includes mass disasters, accidental burning cases and homicidal which may have been exposed to harsh conditions such as heat, arson or

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. Several environmental exposures and allied factors play vital role in degradation of DNA by breaking its molecules into smaller pieces. Hence, it is suggested to use legitimate provisions while collecting and preserving these samples. 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 analyse to determine disparity in the quality and quantity of DNA from direct heat exposed body parts and indirect heat exposed body parts[13].

DNA EXTRACTION

All the samples were collected/ submitted in Forensic Science Laboratory 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 the various DNA extraction techniques such as phenol-chloroform extraction, FTA card, Automate extraction (mainly used for blood samples), Chelex extraction etc. Phenol-Chloroform method is quite sensitive technique used for DNA extraction[14]. Automate extraction is considered as one of the valuable methods which is used for the degraded samples or samples that has faced high temperature.

For DNA analysis, extraction and purification is primarily performed. For extraction, rupturing of cell and nuclear membrane is crucial to release DNA in the solution. Buffer solution and Proteinase K solution are used to rupture the cell 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 analysed by quantification1[5]. 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 analysis1[6].

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. 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 number of 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 machine1[8].

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 ("amplicon") at each cycle of the PCR[19,20]. 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. For each sample, an "amplification curve" is retrieved as a result.

The quality and 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 (OD 260/280) provide an estimation of purity of DNA. OD280 is corresponding to protein content[21]. The concentration of extracted DNA can be calculated from optical density at 260 nm.

RESULT AND DISCUSSION

In forensic cases such as burnt dead bodies (homicidal, suicidal, fire and arson cases etc), it is most challenging to establish the identity of an individual. In such cases, forensic experts collect the body tissues, bones/ teeth to generate DNA profiling and determine the identity of victim. In such cases, the burnt tissues of muscles are recovered in dark brown/ blackish colour and fibres are collected from the lower side of burnt material. When DNA was quantified, obtained quantity of DNA was very less/ negligible in most of the samples. During this analysis, it was observed that Large autosomal target was most effected part which could not be amplified during RT PCR amplification. The quantitative analysis of DNA in indirect heat exposed dead bodies and direct heat exposed bodies are given below in table no.1 & table no. 2-

Sample No.	T. Large autosomal (DNA in ηg./µl.)	T. small autosomal (DNA in ηg./µl.)	Y- (DNA in ηg./µl.)
1	40.68	55.21	83.56
2	30.56	32.36	52.96
3	17.26	18.96	20.32
4	20.18	39.24	56.18
5	34.67	52.98	48.16
6	43.12	41.04	69.87
7	21.23	57.16	83.12
8	19.89	49.31	51.23
9	28.94	42.34	46.98
10	15.67	29.13	67.17
11	21.49	18.72	38.60
12	24.23	27.20	34.51
13	34.19	17.89	70.65
14	20.06	25.14	38.19
15.	41.13	20.84	43.45
Average	**27.555**	**35.168**	**53.663**

Table No. 1; Quantitative analysis of DNA from indirect heat exposed bodies.

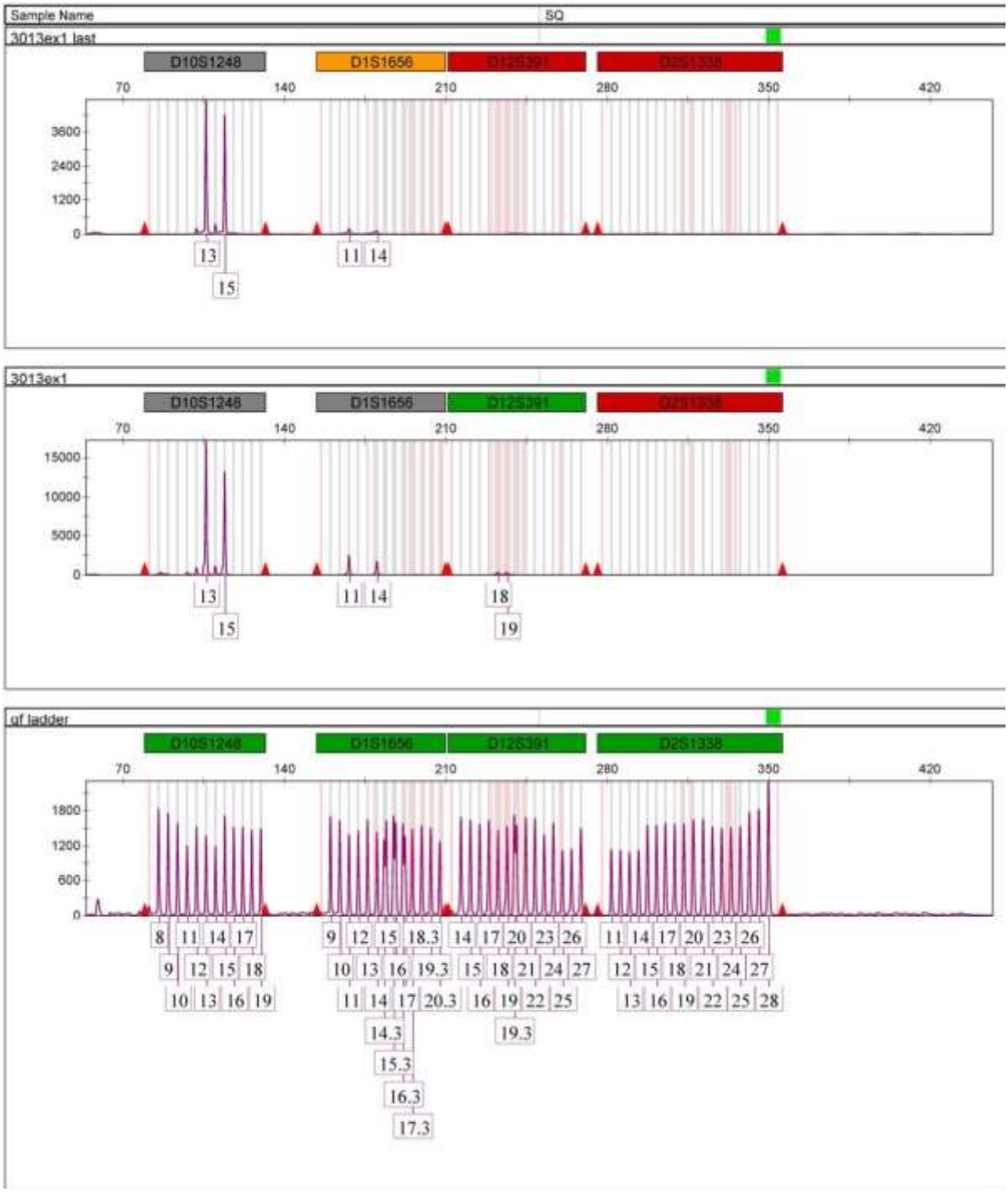
Sample No.	T. Large autosomal (DNA in ηg./µl.)	T. small autosomal (DNA in ηg./µl.)	Y-(DNA in ηg./µl.)
1	-	10.76	9.76
2	-	13.25	2.76
3	-	6.23	5.97
4	-	13.21	3.38
5	-	8.17	8.13
6	0.00	00	00

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
Average	**0.026**	**5.599**	**4.073**

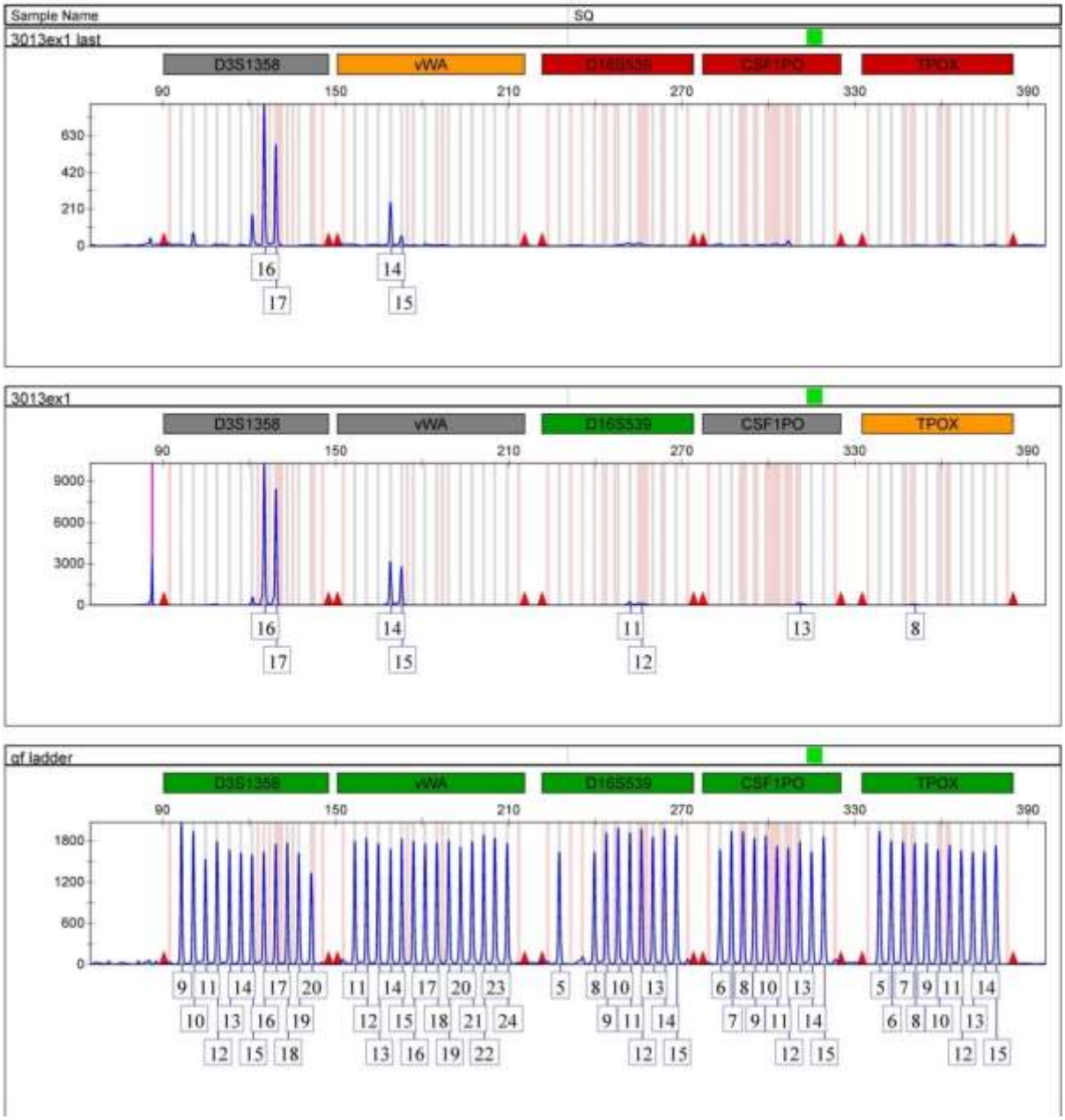
Table No. 2; Quantitative analysis of DNA from direct heat exposed bodies.

However, the small autosomal targets were less effected as there was no direct heat. Hence 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 0.026 **ηg./µl** while average quantity of small targets was 5.599 **ηg./µl** and Y DNA was 4.073 **ηg./µl.** By following same way, DNA profile generated from the indirect heat exposed dead bodies/ contact with fire provided hight quantity of DNA. In indirect heat exposed dead bodies, the Large autosomal targets were 27.555 **ηg./µl** while the quantity of small targets were 35.168 **ηg./µl** and avg. quantity of Y DNA was 53.663 **ηg./µl.**

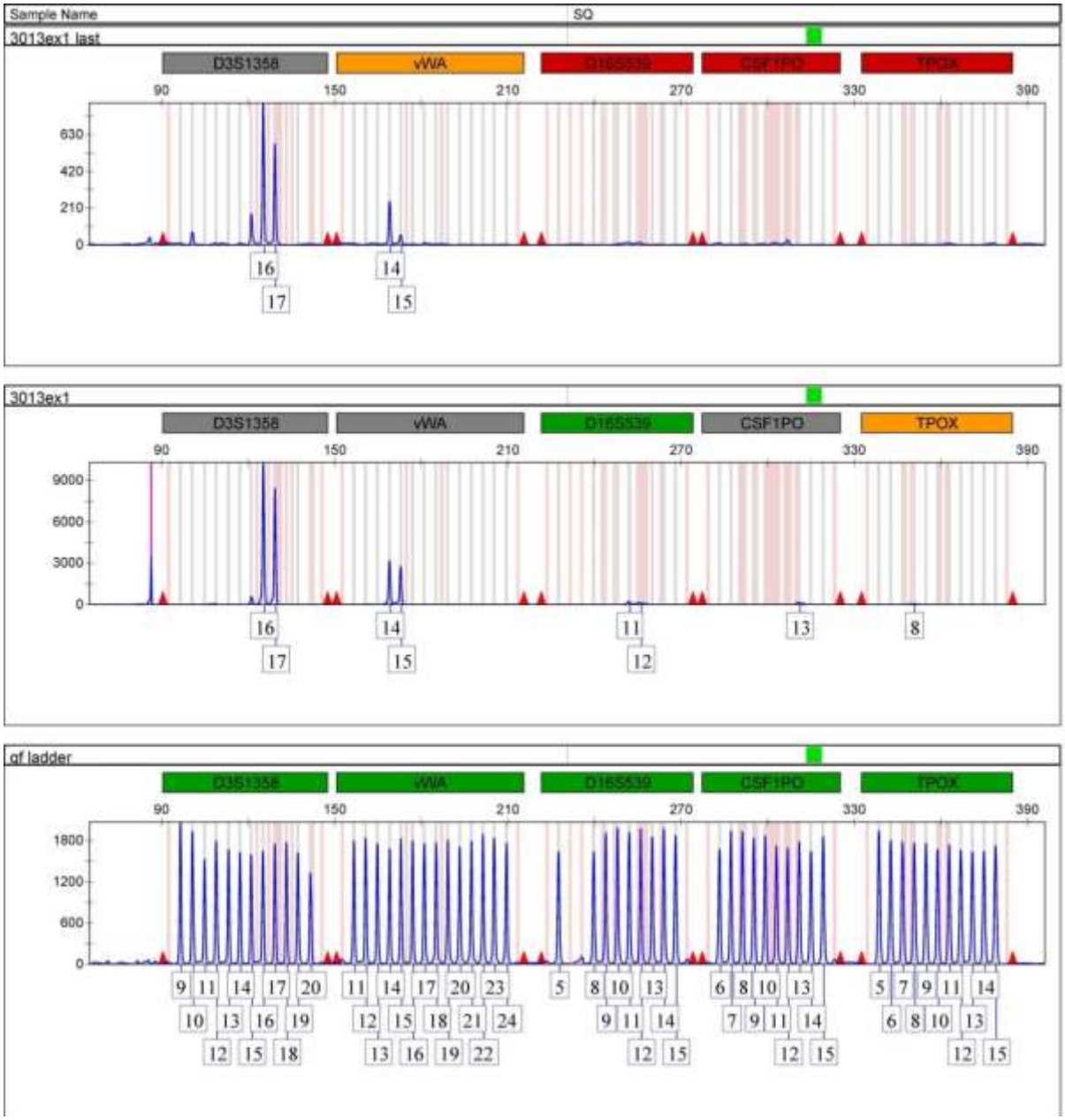
The results of this study suggest that in burnt cases (direct heat), partial DNA profile will be generated. It is also observed that if there is burnt tissues/ body parts have been found on the spot, the outer burnt portion should be removed from the surface and lower portion of muscles/ tissues should be taken for DNA profiling. The generated DNA profiles are given below in electropherogram no.1, 2 & 3-



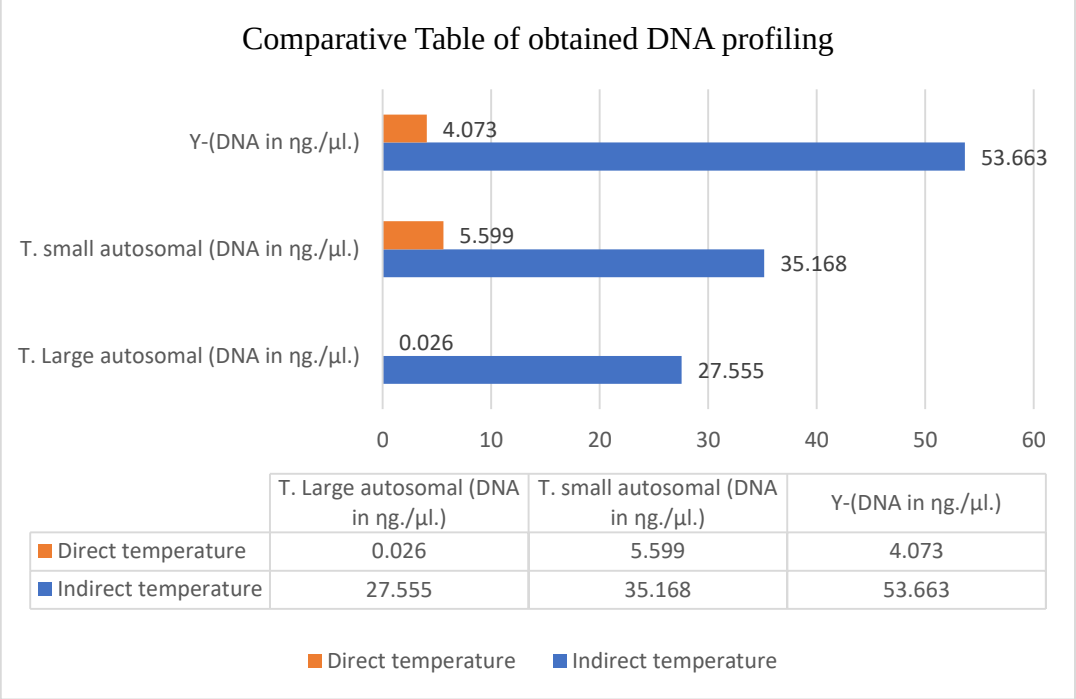
Electropherogram no.1; DNA profiling obtained from the tissues of direct heat exposure.



Electropherogram no.2; DNA profiling obtained from the tissues of direct heat exposure.



Electropherogram no.3; DNA profiling obtained from the tissues of indirect heat exposure.



Graph no. 1; Graphical presentation of comparative table of obtained DNA profiling.

A comparative graphical presentation of DNA profiles generated from direct heat & indirect heat exposed dead bodies in graph no.1. In all samples STR profiles were analysed that were devoid of PCR artefacts. Removal of inhibitor was found effective in isolating the high-quality genomic DNA. This study suggests that in direct heat exposed 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 noticed due the impact of fire.

It is very important to obtain appropriate quality & quantity of DNA to establish identity from the putrefied and unidentified dead bodies or burnt dead bodies. The study suggests that direct fire affected area of body tissue should not be taken for DNA analysis. The soot does not affect the DNA profiling hence such area may be used for DNA profiling.

CONCLUSION

In forensic science, DNA profiling facilitates forensic experts to establish identity of deceased person from various types of ruminants. Most often perpetrators deliberately set fires to destroy dead bodies and evidences to hide identity of victims. In such cases, DNA has provided its feasibility in identification of unidentified dead bodies or burnt cases. Some existing studies suggests that extreme heat on blood will affect DNA profiling. As blood is considered prime source of DNA that will be no longer available traceable after an exposure to a temperature of 1000°C. Therefore, it becomes a necessity to obtained appropriate quality & quantity of DNA for identification from the putrefied and unidentified dead bodies. STR profiles that are devoid of PCR artefacts and removal of inhibitor are found effective in isolating the high-quality genomic DNA. Now a day, the use of DNA to identify the victim from the human remains after an accidental/deliberately fire, arson fire or mass disaster has become a standard technique in the scientific community. 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.

ETHICAL CONSIDERATION: NA

CONFLICTS OF INTEREST: NA

SOURCE OF FUNDING: Forensic Science Laboratory.

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