Research Journal of Forensic Sciences ISSN 2321–1792
Vol. **7(2),** 8-14, July **(2019)** *Res. J. Forensic Sci.*

The effects of heat and molotov incendiary device fluids on DNA analysis

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Available online at: www.isca.in, www.isca.me
Received 18th June 2019, revised 10th July 2019, accepted 25th July 2019

Abstract

Using the findings from terror attacks such as hand-made incendiary devices, the chemical constituency of the explosive can be elucidated to identify the terrorist group responsible for the attack. It is also possible to clarify the incident by analyzing the DNA from the fingerprint. The purpose of this study is to obtain DNA from the fingerprint found on the explosive at the crime scene and to analyze the thermal effect of the DNA on profiling. Fingerprint samples were put on glass, paper and metal sheet surfaces and were individually exposed to diffrent heat conditions for 3 minutes. A total of 108 samples were subjected to DNA analysis using GlobalFiler® PCR Amplification Kit and were typed using the ABI 3130 electrophoresis device. From the samples, complete DNA profile was obtained at 50°C and 90°C on the paper surface and at 50°C on the glass and metal surfaces. On all surfaces and at all temperatures, the gender-identifying locus, amelogenin, was able to be observed. This study, which is conducted for the first time in Turkey, may significantly contribute to the identification of attackers and clarification of the events particularly in terror acts.

Keywords: Crime scene, explosions, fingerprint, molotov events, forensic science.

Introduction

Explosion incidents leave significant effects on the population in economic, psychological and sociological terms, causing irreversible harm. At this point, the duty of the state is to ensure that the offenders are caught as soon as possible, thus, to ease the public conscience, to have them brought to justice and punished as soon as possible. The incendiary known as the "Molotov bomb" is prepared by mixing some sulfuric acid and gasoline-paraffin mixture and is ignited with a fuse. The device incinerates the target. It is dangerous for the safety of life and property, causing disruption of the public order. Using the findings at the crime scenes from the terrorist attacks, the type of the explosive can be identified. Moreover, fingerprint analysis can also be run on the said findings[1-3].

However; in terror attacks where explosives and hand-made incendiary materials are used, the findings are exposed to heat, which requires the reassessment of the conditions under which DNA can be obtained. Hence, analysis of the evidence found during the criminal investigation is indispensable not only to find the fingerprints but also to process genetic identification in order to find the perpetrator[4,5]. As is the case with each transfer made by touching, there are variables determining the amount and quality of the cells left, thus the DNA; such as the intensity, duration, the surface on which it is left, the characteristics of the individual leaving the print, the scene of the print, the time passed until the evidence has reached the laboratory[6-8].

The purpose of this study is to obtain DNA from the fingerprints collected from the explosive surface remains in explosions where hand-made incendiaries were used and to investigates the thermal effect on DNA analysis to determine its potential effects on DNA analysis.

Materials and methods

Sample Collection: A total of 108 fingerprints samples were taken from six informed and consenting voluteers between the ages 26-50, consisting of 3 women and 3 men.

The fingerprint development process was carried out in Istanbul Provincial Directorate of Crime Scene Investigation Branch Fingerprint Development Lab and the genetic analyses were made in Istanbul University Institute of Forensic Medicine, Forensic Molecular Genetics Lab. This work was supported by Istanbul University Scientific Research Projects Unit (Project Number: 56843).

Preparation of Samples: Voluteers were asked to provide fingerprints by contacting model materials for 10 seconds. All paper was used to model paper, whereas galvanised steel metal and microslides were used to model metal and glass surfaces, respectively. The obtained fingerprints were stored for 3 months and scenes consistent with expectations from a trace scene were constructed.

For each research participant; a total of 108 samples were taken. Samples numbering 18 were taken from the fingerprints exposed to heat only; 18 samples were taken from the fingerprints left on surface, contaminated with molotov fluid and exposed to heat; and 18 samples were taken from the fingerprints left on the surface 1 minute after being immersed in molotov fluid and exposed to heat. As for the control group, the buccal were obtained.

DNA Analysis: The DNA analysis process of the study consists of six phases, namely; the collection of swab samples from surfaces, DNA isolation, determination of the amount of DNA, PCR phase, electrophoresis of PCR products in ABI PRISM ® 3130 genetic analysis device and analysis of the post-electrophoresis data.

Collection of Swab Samples from Surfaces: Swabs immersed in double-distilled water were used to collect samples from the surfaces used in this study.

DNA Isolation and Quantification: DNA isolation was performed using silica-based QIAamp® DNA Micro Kit (Qiagen) commercial kit. Quantitation of DNA isolates was performed using Qubit® fluorometer with Quant-iTdsDNA HS (High Sensitive) Assay kit (Invitrogen).

PCR Analysis: DNA samples isolated by GlobalFiler® PCR Amplification Kit (Life Technologies) were amplified using Gene Amp 9700 (Life Technologies) instrument. PCR parameters were as follows: incubation at 95ºC for 1 minutes; denaturation at 94ºC for 10sec., annealing and extending at 59ºC for 90sec., (for 29cycles) and final extension 60ºC for 10 minutes.

Electrophoresis typing: A four capillary ABI PRISM® 3130 Genetic Analyzer (Life Technologies) electrophoresis device was used in this study. A mixture of 0.4µl of GeneScan™ 600 LIZ® Size Standard (internal standard) and 9.6 Hi-Di Formamide was prepared for each sample. The PCR product (1µl) and the formamide size standard mixture (10µl) were added to each well. MicroAmp® Optical 96-Well Reaction Plate containing the mixtures was placed in the device. Electrophoresis of the samples was carried out in 36cm capillaries and using polymer POP4 (Life Technologies).

Data analysis: The raw data obtained from the samples at the post-electrophoresis stage were displayed, typed and evaluated using GeneMapper® ID-X software v1.4 (Life Technologies) analysis program. Informative profiles were recorded as >24 alleles called (plus Amelogenin) that match the donor.

Results and discussion

A negative control was used to check the presence of contamination in studies conducted with the GlobalFiler® PCR Amplification kit. No amplification was observed in the negative controls. In order to ensure proper function and adequate accuracy of the GlobalFiler® PCR Amplification kit, the positive control available in the kit was compared with the profiles available in the control kit. The mean and standard deviation of the RFU values of the highest peaks obtained from all samples were calculated. Analysis threshold values were defined to be 50 RFU by calculation. The standard deviation for allele peak sizes of all samples was calculated for repeatability study and the means of the standard deviation values were found for all loci. The mean value obtained from the averages of the standard deviations for all loci was 0.356.

According to the results achieved after processing in three phases, a complete profile suitable for comparison was obtained from the paper surface at 50°C and 90°C and a partial profile was obtained at 110°C and 150°C. As for the glass and metal surfaces a complete DNA profile suitable for comparison was obtained at 50°C and a partial profile was obtained at 90°C and 110°C. While partial profile was obtained from glass and metal surfaces at 150°C, no profile was obtained from the fingerprint samples which were contaminated with molotov fluid and then exposed to 150°C heat. From all surfaces exposed to different conditions, no DNA profile suitable for comparison was obtained at 200°C and 300°C. Gender specifying locus, amelogenin, was observed on all surfaces and at all temperatures.

The GlobalFiler™ Kit used consisted 24 regions (D7S820, D5S818, CSF1PO, D1S1656, D13S317, D2S441, D12S391, D10S1248, D18S51, FGA, D21S11, D8S1179, VWA, D16S539, TH01, D3S1358, AMEL, D2S1338, D19S433, DYS391, TPOX, D22S1045, SE33,6 Y-Indel) and included shorter amplicon regions than other identification kits used in the field.

Analysis of the results showed that amplicons yielding short products were more successful than regions yielding long amplicons, short loci gave more results. Genotyping results obtained from metal, glass and paper surfaces that were exposed to different pre-treatments and temperatures are shown in Table-1 on locus basis and their success rates in forming a complete profile are shown in Figure-1, 2 and 3, also on locus basis. The DNA profile obtained from paper surface exposed to 90°C heat following fingerprint, using GlobalFiler® PCR Amplification kit, is as shown in Figure-4.

Thanks to the rapid developments and the qualified specialists in the struggle against crime and criminals, it has become possible to conduct comprehensive and multi-lateral research on the evidence collected from crime scenes. By determining the type of the explosive or hand-made incendiary material from the evidence collected from the crime scene of the terror act, it is possible to identify the terrorist group responsible for such crimes. One of the important purposes of crime scene investigation is to investigate fingerprints in order to identify the offender. The presence of a fingerprint that could be used for

identification on materials found in crime scene is very important for understanding the crime and apprehending the criminals. In Turkey, approximately 25-30% of the fingerprints collected from crime scenes and brought to the laboratory are reported to be not suitable for comparison[9]. When fingerprint comparison method is insufficient, other evidence from crime scene that may contain DNA are used.

Table-1: Number of loci expected and observed for the DNA profiles obtained from different surfaces that were exposed to different conditions

Temperature			50°C	90°C	110°C	150°C	200°C	300°C
Surface	Treatment	Number of Loci Expected	Number of Locus Observed					
Paper	Heat treated after fingerprint	24	24	24	15	11	1	1
	Immersed in molotov and heat treated	24	24	24	18	12	1	1
	Molotov and heat treated after fingerprint	24	24	21	11	9	1	1
Glass	Heat treated	24	24	10	6	4	1	1
	Immersed in molotov and heat treated	24	24	13	10	9	1	1
	Molotov and heat treated	24	24	6	5	3	1	1
Metal	Heat treated	24	24	11	7	4	1	1
	Immersed in molotov and heat treated	24	24	13	10	5	1	1
	Molotov and heat treated	24	24	9	6	1	1	1

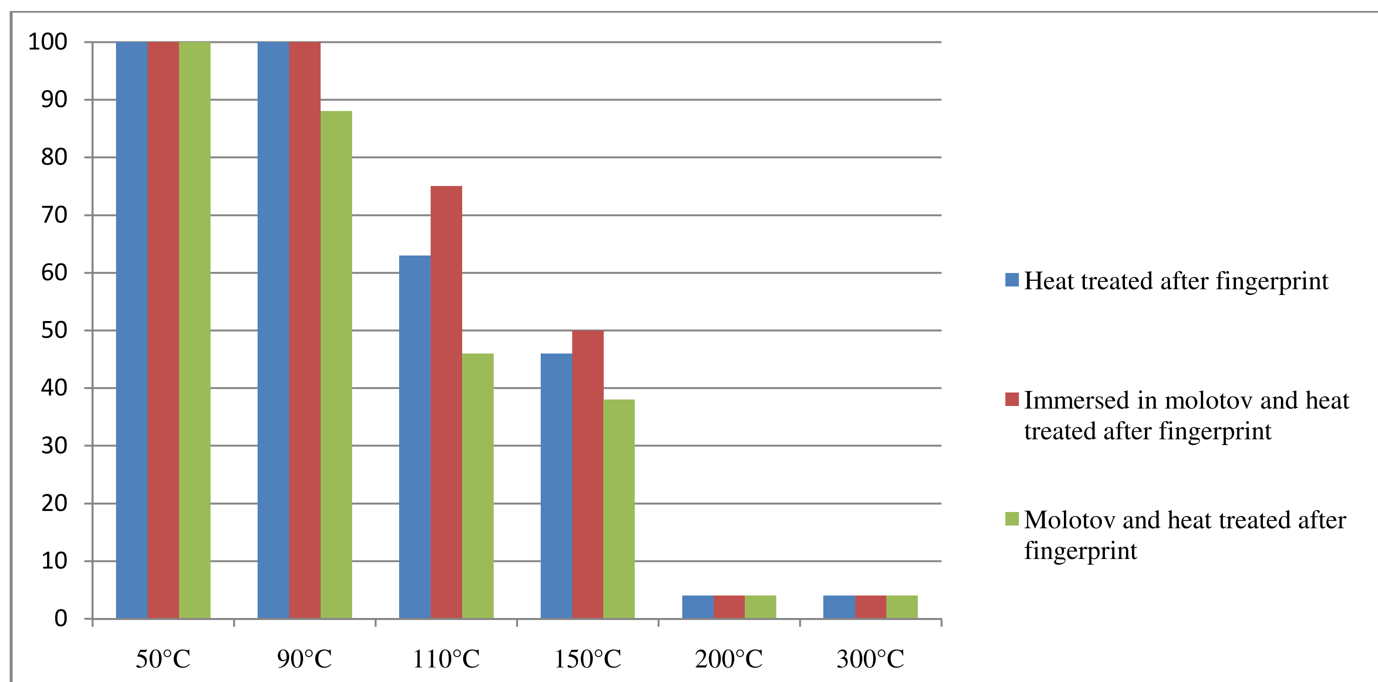

Figure-1: Success rates of the DNA profiles obtained from paper surfaces in forming complete profile on locus basis (Heat treated following fingerprint - Immersed in molotov and heat treated following fingerprint - Molotov and heat treated following fingerprint).

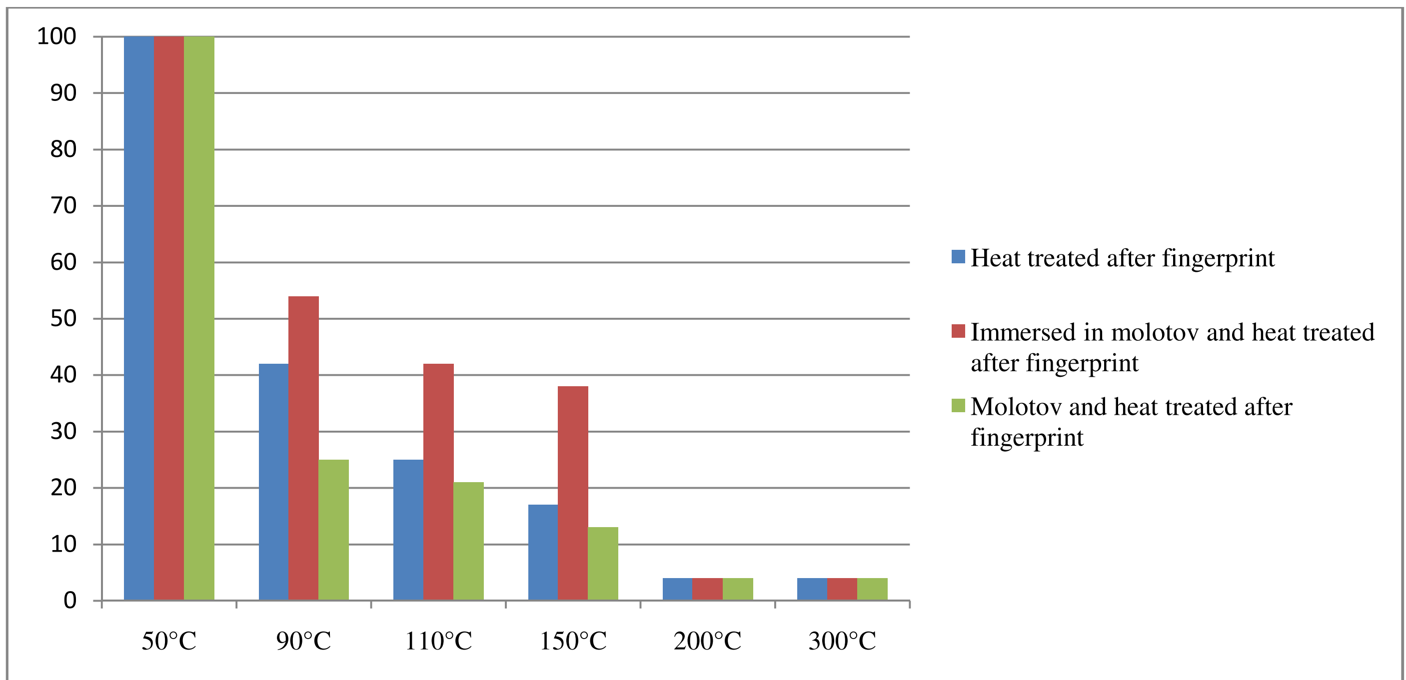

Figure-2: Success rates of the DNA profiles obtained from glass surfaces in forming complete profile on locus basis (Heat treated following fingerprint - Immersed in molotov and heat treated following fingerprint - Molotov and heat treated following fingerprint).

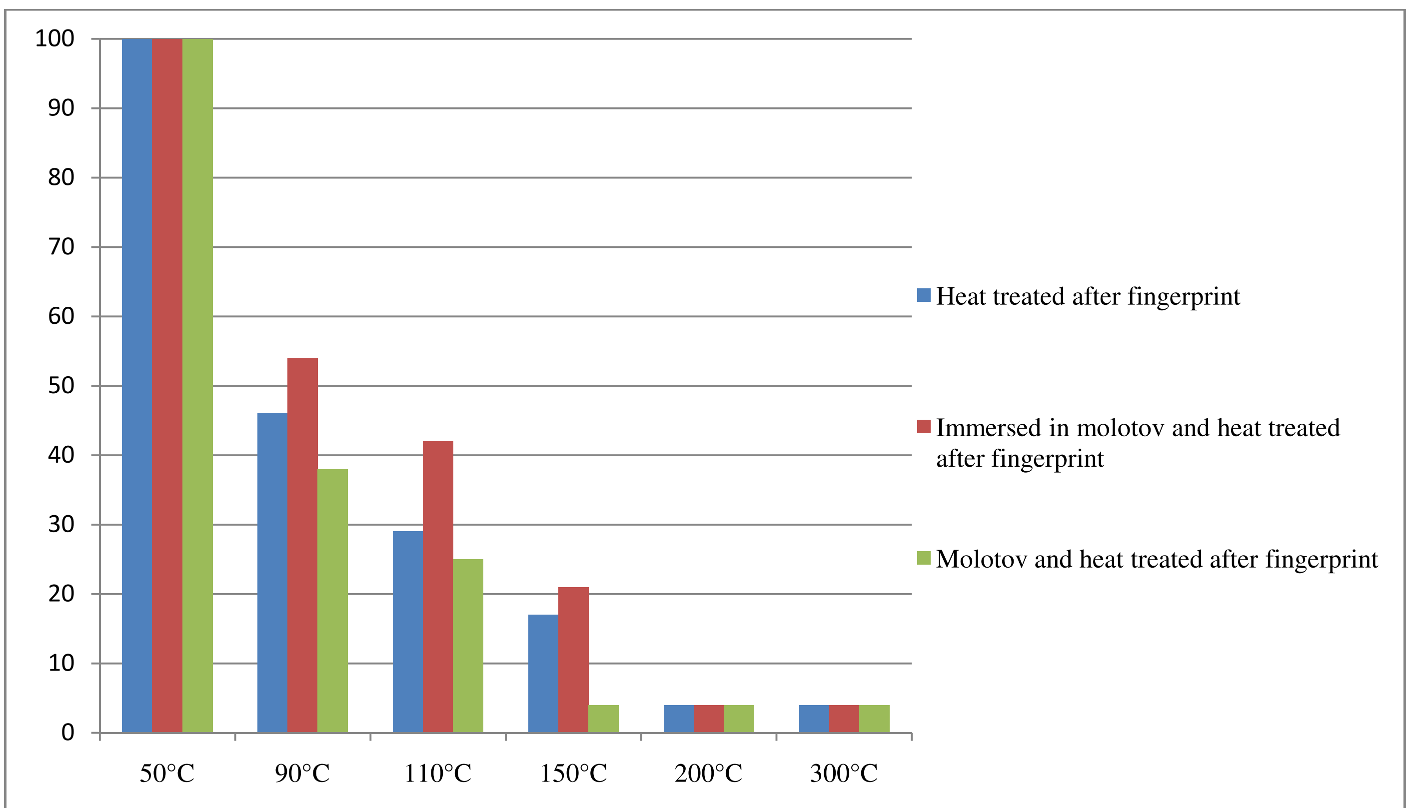

Figure-3: Success rates of the DNA profiles obtained from metal surfaces in forming complete profile on locus basis (Heat treated following fingerprint - Immersed in molotov and heat treated following fingerprint - Molotov and heat treated following fingerprint).

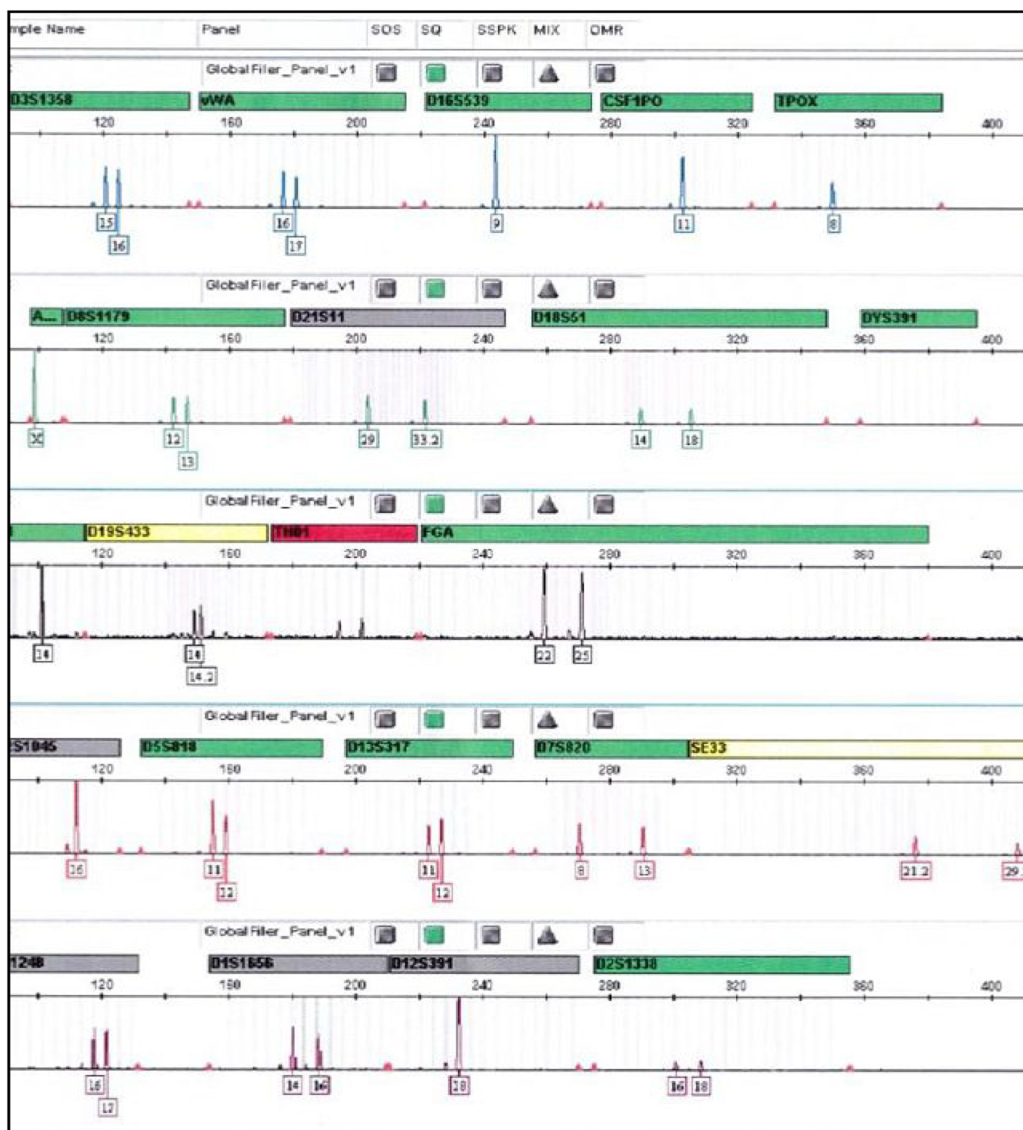

Figure-4: DNA profile of the sample obtained from paper surface exposed to 90°C heat.

Glass, metal and paper surfaces were used in this research as they are commonly used in daily life, they can be easily supplied and they are the surfaces where the molotov fluid and explosive mixtures may come into contact. Comparison of the surfaces showed that glass surfaces gave the best results for the development of fingerprint suitable for comparison. Metal and paper surfaces followed, respectively. According to the studies reporting that glass surfaces give much better results, the porous structure of the surface increases perspiration rate. This both ensures clear papilla lines and enables deposition of a greater amount of epithelial[10].

Temperature can adversely affect the development of the resultant fingerprint. Temperatures above 200°C are sufficient to destroy the organic compounds of the fingerprint[11,12].

While the temperature variable was the same in all conditions, fingerprint development results changed depending on the environmental conditions, which may be due to contamination[13]. It was seen in the study that, an oily layer was formed on surfaces immersed in molotov fluid, which had positive effect on the development of the fingerprint on the surface. However; contamination of the fingerprints on the surface with molotov fluid had an adverse effect on the permanence of fingerprints.

In the study conducted by Shelef et al. using different molotov components, it was determined that gasoline and diesel fuels formed a protective layer on the surface. It was proven that fingerprints left on surfaces contaminated with these easily available types of fuels, gave high quality results[14].

To the best of our knowledge; there is no literature study resembling ours but the effect of the chemicals used in the development of fingerprints and the effect of different lengths of light were mostly discussed. In the experimental research conducted by Kumar et al., the fingerprint was developed on blood stain using various chemicals (Rodamine 6G, ninhydrin, crystalline violet and silver nitrate etc.) and STR analysis was performed on the DNA obtained and it was reported that the use of silver nitrate in particular reduced the DNA amount that could be harvested[15].

In another study conducted by Gardner S.J. et al., the effect of different temperature parameters on the development of fingerprint was investigated and results were reported to be obtained on different surfaces at 300^0 C, but no relevant DNA analysis was made[16].

Ostojik et al. analyzed the effect the duration of permanence on various surfaces had on profiling by performing STR analysis on 900 fingerprint samples. According to the results they found that more than 50% of the profile could be identified from fingerprints up to 40 days. They also found that the profiles obtained from glass surfaces were better than plastic and paper. The fact that our results do not sufficiently overlap with the findings obtained by Ostojik et al. in their STR analysis, is assumed to be due to the success of the GlobalFiler™ kit in identification, thanks to its utilisation of short amplicon regions[4].

Whether identification was made or not, the fingerprints samples from potential crime scenes were exposed to DNA analysis in our study. In this way, the temperature ranges and the conditions in which DNA could be obtained from the organic molecules and epithelial cells in the fingerprint were determined. The results achieved according to these findings showed that; the paper, glass and metal surfaces which were exposed to three phases and then sampled, gave complete profiles suitable for comparison at 50^oC, whereas the paper surface (treated with heat following fingerprint and immersed in molotov fluid and treated with heat following fingerprint) gave complete profiles suitable for comparison at 90^oC. On all surfaces and at all temperatures, the gender-identifying locus, amelogenin, was observed. All analysis results showed that amplicons yielding short products were more successful than amplicons yielding longer products, short locus gave better results. This finding overlaps with the findings of Opel et al.[17].

Conclusion

Thanks to the rapid developments and the qualified specialists in the struggle against crime and criminals, it has become possible to conduct comprehensive and multi-lateral research on the evidence collected from crime scenes. One of the important purposes of crime scene investigation is to investigate fingerprints in order to identify the offender. We believe that, this study, which is conducted for the first time in Turkey, could significantly contribute to the identification of attackers and clarification of the events particularly in terror acts.

Acknowledgements

We would like to thank the Istanbul Provincial Directorate of Security and Istanbul University Scientific Research Unit for their support in this study.

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