Basic Medical Research

DNA quality and quantity in adipose tissue: a comparison of the effects of bomb explosion

Leonardo[1], Ade Firmansyah Sugiharto[2], Wresti Indriatmi[3], Djaja Surya Atmadja[2], Ahmad Yudianto[4], Herkutanto[2], Wahyu Widodo[5]

pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.oa.247206
Med J Indones. 2023;32:205–11

Received: October 11, 2023
Accepted: January 05, 2024
Published online: February 07, 2024

Authors' affiliations:
[1]Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [2]Department of Forensic and Medicolegal, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [3]Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [4]Department of Forensic and Medicolegal, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [5]Indonesian National Police, Batam, Kepulauan Riau, Indonesia

Corresponding author:
Leonardo
Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jalan Salemba Raya No. 6, Central Jakarta 10430, DKI Jakarta, Indonesia
Tel/Fax: +62-21-31930373, +62-21-31930371, +62-21-3912477/ +62-21-3912477
E-mail: dokterleonardo@gmail.com

ABSTRACT

BACKGROUND Adipose tissue is often overlooked in DNA testing due to misconceptions about its DNA content. However, its shock-absorbing qualities may be useful for high-pressure scenarios like bomb blasts. This study aimed to evaluate DNA quality and quantity in adipose tissue affected by blasts compared to that in unaffected tissue.

METHODS 10 adipose tissue samples were taken from regions near and far from the blast, representing the blast-exposed and non-blast-exposed groups. The adipose tissue was stored at a low temperature for 5 days, after which an organic extraction method was applied. The purity of the DNA extract was assessed using a NanoDrop spectrophotometer, and its integrity was evaluated using 0.8% concentration gel electrophoresis at 60 V for 90 min. DNA typing was conducted using the GlobalFiler™ kit, and DNA quantity was determined with the Quantifiler™ Trio DNA Quantification kit.

RESULTS Of 20 DNA extracts from adipose tissue, all samples demonstrated purity, integrity, and complete typing results. Adequate integrity was found in 90% of samples in both groups. A 50% incidence of allele shifting was observed at the D7S820 locus within the blast-exposed group.

CONCLUSIONS DNA from blast-exposed adipose tissue exhibited no significant quality or quantity differences from non-blast-exposed tissue. This suggested adipose tissue's potential as an alternative DNA source in a bomb explosion.

KEYWORDS adipose tissue, bombs, DNA typing, human identification

A bomb blast examination is primarily performed for accurate and rapid personal identification, enabling the prompt identification of victims. The victims' body parts are often found scattered, especially at the center of the blast.[1] The body parts found are usually skin and adipose tissue, which comprise 14–20% and 20–26% of the human body composition in men and women, respectively.[2] This complicates the examination process, and identification cannot be determined using standard methods. Using base pairs (bp) in nucleus DNA as a primary identification marker has played a major role in identifying victims since the 9/11 bomb tragedy at the World Trade Center.[3] The Federal Bureau of Investigation established a combined DNA index system as a guide to determine the loci using short tandem repeat analysis to be compared directly or indirectly for identification.[4] Research on DNA typing in samples affected by bomb explosions using triacetone triperoxide (TATP) explosives, which are unstable and highly volatile,[5] has never been conducted before.

Despite this, TATP is frequently used by terrorists due to its ease of manufacture, making it the signature type of bomb for various terrorist groups.[6]

Identification using DNA begins with selecting biological materials that are considered feasible and have DNA content.[3] Adipose tissue has not been studied for primary identification in forensics because of the significantly lower quantity of DNA in adipose cells compared to other tissues. For example, in muscle tissue, the DNA quantity is 4 times higher than in adipose tissue.[7] However, cells in adipose tissues have large cytoplasms and fat globules, which push the cell's nucleus to the periphery. Further, these tissues are located beneath the skin, serving as padding and protecting the underlying tissues and organs,[8] which could protect the DNA from the effects of excessive pressure. Following sample selection, the specimens are preserved under optimal conditions to prevent decay or cellular degradation.[9]

Preservation methods are classified into mechanical, chemical, and physical protections. Mechanical protection, such as aluminum foil, plastic containers, and paper, protects the DNA from the external environment and cross-contamination. Chemical protection provides an internal environment for the DNA, such as a buffer. Physical methods such as drying or temperature control are beneficial for reducing the metabolism of cells and bacteria, thereby hindering the degradation process. Preserving soft muscle tissue samples using a temperature of −4°C successfully maintains alleles until Day 7[9] and a temperature of −20°C until Day 365.[10] Cold temperatures as a DNA preservation medium slow cell degradation by creating non-ideal conditions for the growth of decomposing microorganisms, decelerating chemical reactions, and reducing the activity of degrading enzymes.[9,10] These methods can be combined, for example, using an ice box for mechanical protection, where samples are placed in a cold buffer. There are no recommendations from the International Society for Forensic Genetics regarding the use of DNA preservation media. However, DNA preservation is considered effective for soft tissue and is the gold standard for temperature control and dehydration.[11] DNA extraction is carried out to obtain the DNA from nucleated cells free from contaminants. Extraction by the organic method (phenol-chloroform) is the gold standard, although other easier modifications or kits are available.[12] However, organic extraction is avoided because it is time-consuming and involves toxic substances.[3]

In this study, we hypothesize that there is no difference between the DNA of blast-affected adipose tissues and that of non-blast-exposed adipose tissues. Therefore, this study aimed to evaluate the quality and quantity of DNA in adipose tissue affected by blasts compared to that in unaffected tissue.

METHODS

Study population and design

This study was conducted from December 2022 to March 2023 at Bhayangkara Tk. II Sartika Asih Hospital, Forensic Laboratory Center of Criminal Investigation Agency of the Indonesian National Police, and Laboratory of Genomik Solidaritas Indonesia, and was approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia (No: KET-1378/UN2.F1/ETIK/PPM.00.02/2022). This quasi-experimental study used adipose tissue samples of individuals involved in a suicide bomb explosion in Indonesia, where TATP was used.

The DNA samples were divided into high-pressure explosion (blast-exposed) and minimal exposure (non-blast-exposed) groups. Each group comprised 10 adipose tissue samples. Data from each sample were collected through sample preservation, adipose tissue DNA extraction, spectrophotometric examination, and gel electrophoresis results on adipose tissue DNA extracts.

Sampling technique

This study used the non-probability sampling technique, particularly the consecutive method, in cadaver adipose tissue. The adipose tissue for the blast-exposed group was taken from the unburned waist of the perpetrator, whereas that for the non-blast-exposed group was taken from the inner thigh fat, which was protected from the source of the blast by the skin, muscle, and bone layer. Samples were collected manually from the adipose tissue under the skin using a sterile scalpel. The tissue was separated and cut into 1.5–2 cm × 1.5–2 cm pieces, with a total of 10 pieces for each group.

Sample preservation

Each sample was inserted, labeled, and placed in an ice box at 0–6°C for 5 days. The duration of preservation

was determined based on the typical time span for shipping samples from the blast site to a DNA testing laboratory in Indonesia.

Extraction procedure

Chunks (100 mg) were obtained from each sample. Extraction was performed using the organic extraction method with additional washing with chloroform. DNA extracts were resuspended with 100 µl of ethylenediaminetetraacetic acid (EDTA) or tris-EDTA (TE) buffer.[12]

DNA quality

DNA purity was examined using a NanoPhotometer® N50 (Implen, Germany). A 1.5 µl extract was dropped onto the optical lens using a micropipette. The optical lens was cleaned before the next sample was dropped. Spectrophotometer readings were based on the 260 nm wavelength rather than the 280 nm wavelength. The results were considered good if the comparison ratio was in the range of 1–2.[13]

Gel electrophoresis was performed using a 0.8% agarose gel with GelRed (Biotium, USA) staining in 32 wells. Extracted DNA was diluted to a limit of 200 ng/µl using the TE buffer. The results were read using a GeneRuler 1 kb Plus DNA Ladder (Thermo Fisher Scientific, USA) with a maximum ladder length of 20,000 bp on the left and right ends of the gel to determine the integrity of the DNA. An agarose gel containing the DNA extract and a ladder, stained with TriTrack DNA Loading Dye (Thermo Fisher Scientific), was then subjected to a voltage of 60 V for 90 min. This process ensured that the DNA migrated effectively through bands formed above the top line of the ladder (20,000 bp), indicating high molecular weight (HMW) DNA or that it was not degraded. Bands that widened toward the end of the ladder indicated a size smaller than 20,000 bp, representing a smear (DNA degradation).[3] Results were considered satisfactory if there was HMW and a minimal smear.

DNA typing was performed using a GlobalFiler™ kit (Thermo Fisher Scientific), with 24 loci comprising 46 alleles. DNA extracts were diluted if the DNA levels exceeded the optimal concentration of the GlobalFiler™ kit (0.5–1 ng/15 µl) using TE buffer. A positive control was provided at the start of the examination using DNA control 007, and a negative control was provided using DNA-free distilled water. Results were read using GeneMapper™ ID-X software (Thermo Fisher Scientific™) on a computer connected to the polymerase chain reaction (PCR) machine. DNA typing was performed based on the successful typing of the alleles at each locus examined.

DNA extract quantity

DNA extracts were calculated using the Quantifiler™ Trio DNA Quantification kit (Thermo Fisher Scientific™) and the 7500 Real-Time PCR machine (Applied Biosystem, California). The cycle threshold value was compared with the standard curve to obtain DNA levels.[14] The recorded DNA levels were converted into units of ng/g of adipose tissue, with a cut-off of 1,000 ng/g as the minimum DNA concentration required for performing DNA typing. If the values were above the cut-off value, the DNA quantity was considered sufficient.[15]

Statistical analysis

The chi-square test was employed for statistical analysis when the expected counts were appropriate, which was when <20% of the cells had an expected count below 5, and no cell had a count of zero. In cases where these conditions were not met, Fischer's test was used. The analyses were benchmarked against a 5% significance level ($p<0.05$), leading to the rejection of the null hypothesis. This study also included subgroup analyses to assess the effects of blast exposure on DNA quality and quantity in fat tissue, factoring in various preservation methods and the specific timing of the extraction process. Variables with $p<0.25$ were advanced to a multivariate logistic regression analysis. For each genetic locus, the analysis focused on the most common alleles.

RESULTS

Our findings revealed that the victim's body was divided into three parts because of the suicide bombing event, as illustrated in Figure 1. A median sample weight of 101.17 g with a standard deviation of 0.544 was obtained. The sample in both groups displayed similar results in four aspects: DNA purity, integrity, typing, and quantity (Table 1). There were no significant differences between the blast-exposed and non-blast-exposed groups regarding DNA quality and the four aspects outlined above. The results of gel electrophoresis are shown in Figure 2. Meanwhile,

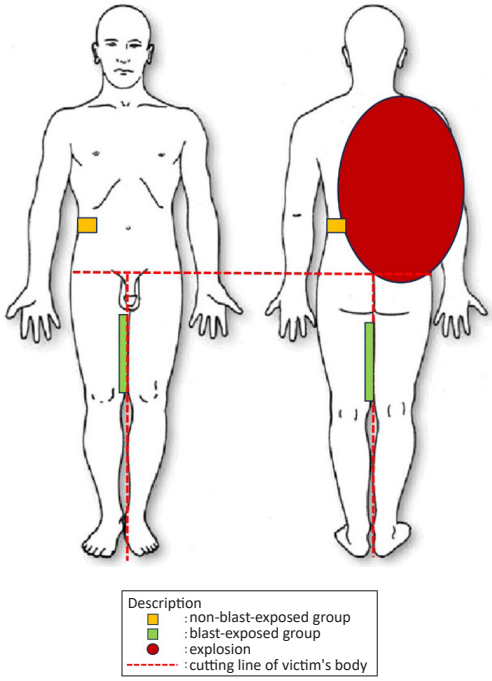


Figure 1. Description of the victim's body and location of sample collection

examples of typing for each group are depicted in Figure 3 a and b. Comprehensive results regarding sample weight, DNA purity, integrity, quantity, and typing are shown in Supplementary Table 1, and the typing of each locus is provided in Supplementary Table 2.

DISCUSSION

We observed satisfactory DNA purity from adipose tissue in a bomb blast case. Two studies successfully extracted RNA from rat[16] and human[17] adipose tissues using an organic extraction method. These results suggest that organic extraction can remove contaminants from the DNA in adipose tissue, preventing interference during further examination, especially during DNA amplification using a PCR machine. During the examination, internal inhibitors such as hematin in the blood and melanin in the tissue and external inhibitors such as humus from the soil may be present.[3]

Adipose tissue has good resistance to the impact of overpressure caused by the explosion of highly explosive bombs. The integrity assessment of DNA in the blast-exposed group showed a 90% result with a good quantity of samples. This study used adipose cells under the human skin, where adipose cells that gather

Table 1. Basic characteristics of the samples

Variables	Groups		*p**	OR (95% CI)
	Blast-exposed, n (%)	Non-blast-exposed, n (%)		
DNA purity			1.00	1.00 (0.018–55.271)
Pure DNA	10 (100)	10 (100)		
Impure DNA	0 (0)	0 (0)		
DNA integrity			1.00	1.00 (0.053–18.574)
Intact DNA	9 (90)	9 (90)		
Non-intact DNA	1 (10)	1 (10)		
DNA typing			1.00	1.00 (0.018–55.271)
Full DNA typing	10 (100)	10 (100)		
Not full DNA typing	0 (0)	0 (0)		
DNA quantity			1.00	1.00 (0.018–55.271)
Sufficient DNA quantity	10 (100)	10 (100)		
Insufficient DNA quantity	0 (0)	0 (0)		

CI=confidence interval; OR=odds ratio
*Fischer test

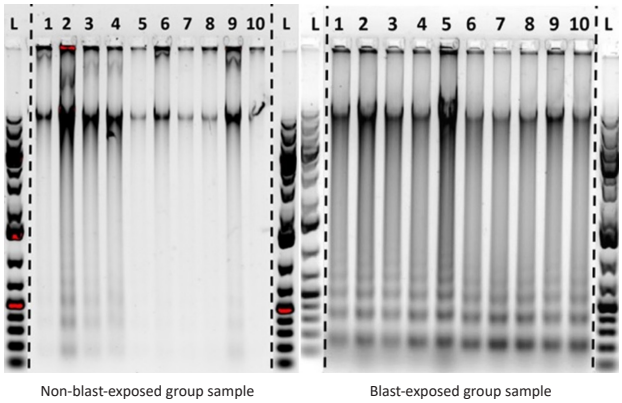
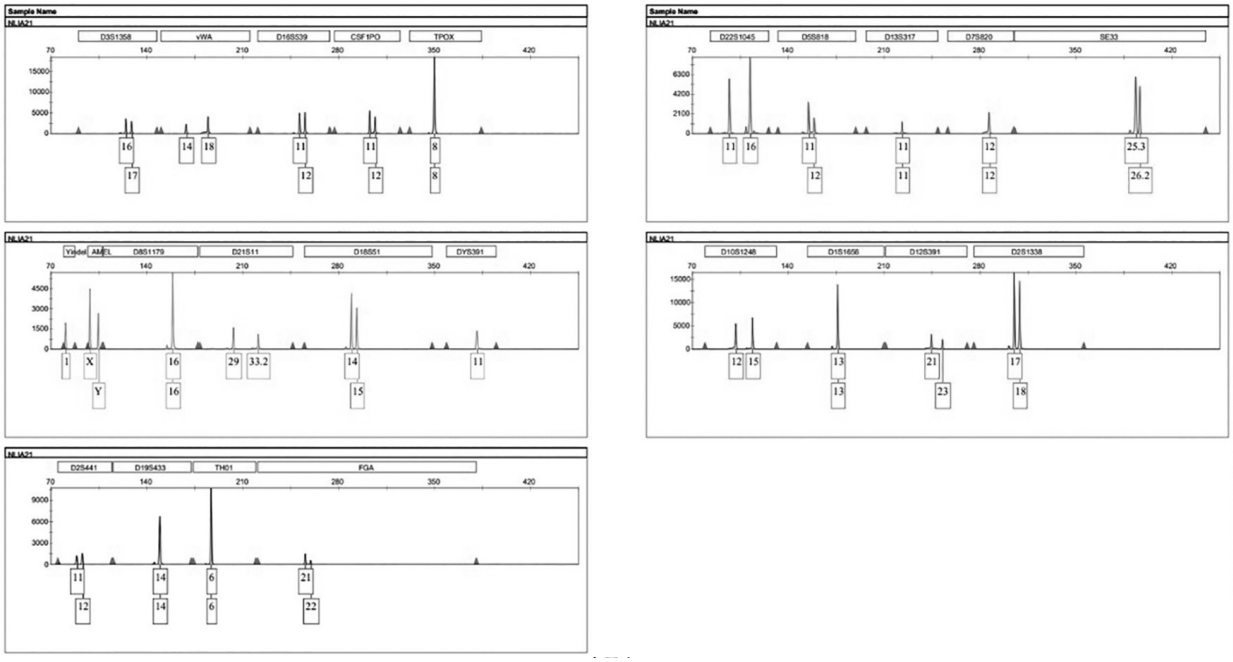


Figure 2. Gel electrophoresis results

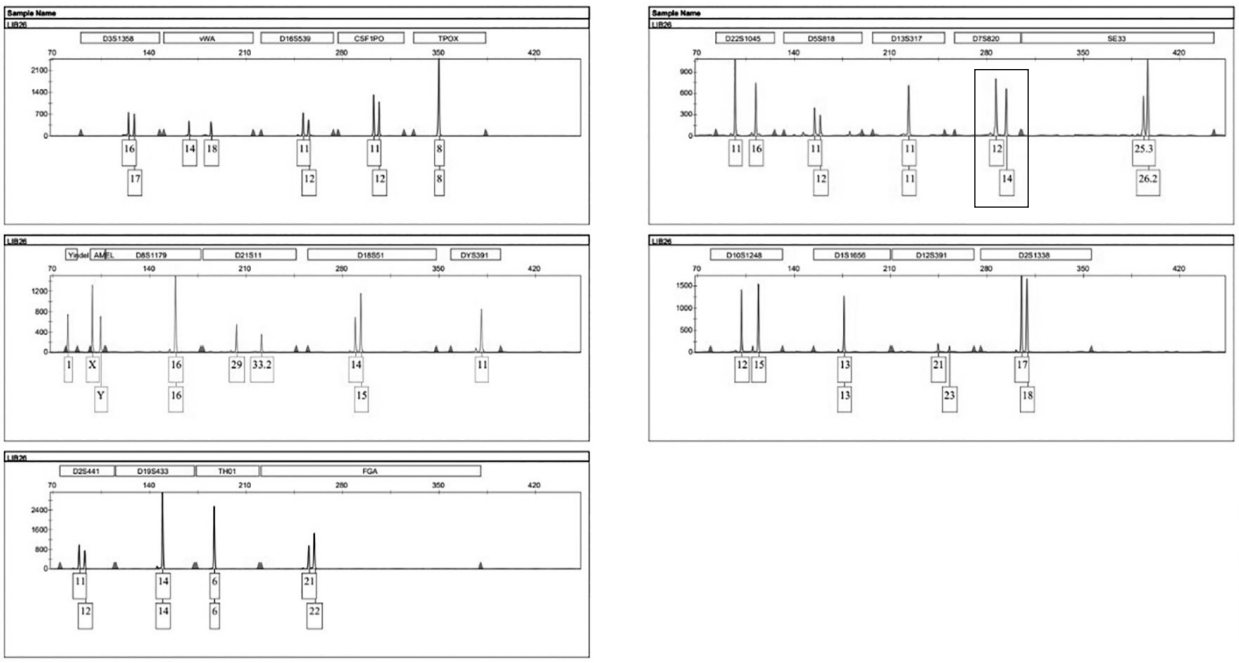
and form adipose tissue transmit external forces and gradually decrease until they disappear according to the biomechanics of trauma. This layer of the body has good thickness, elasticity, and tissue homogeneity, which transmits the force constantly hitting the body. The absence of density differences prevents

Non-blast-exposed group sample no. 1



a

Blast-exposed group sample no. 6



b

Figure 3. DNA typing results. (a) Example of GlobalFiler™ results of the non-blast-exposed group; (b) example of shifting allele (heterozygote 12 and 14 compared to figure a homozygote 12) on D7S820 locus in GlobalFiler™ results of the blast-exposed group (black rectangle outline)

the stress concentration effect (i.e., the area where force distribution occurs because the density of the conducting medium results in a significant increase in stress or force). If the pressure hits dense tissues, such as muscles, bones, and organs with less elasticity, the structure and DNA inside will break.

A study using DNA extracts dissolved in TE buffer and exposed to constant pressure for a specific duration observed that DNA degradation at high pressure increased with prolonged exposure.[18] The different results in this study were attributed to the different sample types, pressure strengths, and

pressure quantities. A bomb explosion produces pressure in two phases. With TATP, the positive pressure reaches 80 kbar, while the negative pressure, caused by the vacuum from the positive pressure, is far below the positive pressure.[5] The adipose cell nucleus, characterized by a large cytoplasm and a loose arrangement of cells in the tissue, is relatively resistant to pressure compared to other body tissues and cells. Consistent with this, the present study found similar integrity and quantity between the blast-exposed and non-blast-exposed groups.

A previous study found a decrease in DNA quantity in the blood that had not been administered an EDTA preservative after a pentaerythritol tetranitrate explosion in a pipe bomb. The results of blood with EDTA preservation with relatively similar pre- and post-blast concentrations[19] are less applicable because, physiologically, blood does not contain EDTA. According to a previous study utilizing DNA touch techniques, DNA quantities in areas close to an explosion, specifically inside the bag, were lower than those in areas more distant from the blast, such as the bag's handle.[20] Previous studies have identified suicide bombers through DNA using sweat and saliva samples, with DNA typing and matching rates of 12% and 48%, respectively.[21] Additionally, a study in 2012 investigating DNA touch samples on a backpack showed contamination of alleles from other individuals (50.9%) and the absence of any detectable alleles (4.7%).[22] To date, further research on the impact of bomb blasts on soft tissue has not been conducted.

The organic method separates the organic phase to the bottom and the aqueous phase to the surface. Adipose tissue contains polar contaminants in the form of lipids that will be completely separated in the organic extraction method.[23] Using organic methods to separate adipose tissue can be challenging due to its high lipid content that can interfere with chemical reactions in the extraction process.[24] Modifying the process by including chloroform washing can help remove inhibitors in adipose tissue.[12] Physical preservation media using cold temperatures can maintain DNA in the adipose tissue qualitatively and quantitatively.

The full typing results for the non-blast-exposed and blast-exposed samples showed no significant differences. In this study, blast-exposed adipose tissues showed an allele shift at the D7S820 locus in five samples (50%). This shift caused the homozygotes (12) to become heterozygotes (12, 14). The D7S820 locus is located on chromosome 7q21 and has a guanine-adenine-thymine (T)-adenine repeat motif and a 0.1% mutation probability. D7S820 is an off-ladder allele located in the flanking region of the DNA. An off-ladder allele does not match the standard reference used by the available kits and is characterized by alleles with odd numbers. In D7S820, the x.3 marker had eight Ts, and the allele with the x.1 marker had 10 Ts. The cause of off-ladder alleles is a variation in the T bases that can stretch during evaluation.[25]

In blast-exposed adipose tissue, alleles may shift owing to contamination or the ejection of different fragments in other directions caused by the casing injection or bomb explosion. Contamination can be prevented by washing with DNA-free distilled water, using personal protective equipment, and sampling skin-protected tissues. These uncontrollable off-ladder alleles are confounding factors in forensic analyses. In this scenario, locus shifts are negligible for up to two loci because each locus has a mutation rate.[3]

This study had several limitations. We did not perform a comparative analysis with other soft tissues, alternative preservation media, or various DNA extraction techniques. Further investigation is required to determine the potential alterations in DNA integrity due to deflagration or thermal effects commonly associated with bomb detonation. Additionally, a structural examination of the DNA was not conducted, which presents an avenue for future research.

In conclusion, adipose tissue was a viable alternative for DNA-based personal identification, including in bomb-blast scenarios. The adipose tissue showed promising results regarding DNA purity, integrity, typing, and quantity in blast-exposed cases. However, further research is needed to compare other tissues, such as muscle, bone, blood, and teeth, and utilize other preservation and extraction methods to identify bomb blast victims.

Conflict of Interest

The authors affirm no conflict of interest in this study.

Acknowledgment

We would like to thank Bhayangkara Tk. II Sartika Asih Hospital, Bandung, for their invaluable support and guidance, Forensic Laboratory Center of Criminal Investigation Agency of the Indonesian National Police for their detailed and meticulous forensic analysis, and Laboratory of PT. Genomik Solidaritas Indonesia for their research contributions. The collaboration, expertise, and dedication of these esteemed institutions have been pivotal in the success of our endeavor, and we are profoundly thankful for their unwavering commitment and support.

Funding Sources

None.

REFERENCES

1. INTERPOL. Disaster victim identification guide - part "A" [Internet]. INTERPOL; 2018 [cited 2023 Mar 1]. Available from: https://www.interpol.int/How-we-work/Forensics/Disaster-Victim-Identification-DVI.
2. Stroh AM, Lynch CE, Lester BE, Minchev K, Chambers TL, Montenegro CF, et al. Human adipose and skeletal muscle tissue DNA, RNA, and protein content. J Appl Physiol (1985). 2021;131(4):1370–9.
3. Butler JM. Forensic DNA typing. 2nd ed. USA: Elsevier Academic Press; 2005.
4. Federal Bureau of Investigation (FBI). Frequently asked questions on CODIS and NDIS [Internet]. Federal Bureau of Investigation (FBI); 2017 [cited 2023 Mar 1]. Available from: https://www.fbi.gov/services/laboratory/biometric-analysis/codis/codis-and-ndis-fact-sheet.
5. Agrawal JP. High energy materials: propellants, explosives and pyrotechnics. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA; 2010.
6. Joint Counterterrorism Assessment Team. Triacetone triperoxide (TATP): indicators of acquisition and manufacture, and considerations for response. Joint Counterterrorism Assessment Team; 2019. p. 10–2.
7. Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Howells GP, Tipton IH. Report of the task group on reference man. In: International Commision on Radiological Protection No 23. Oxford: Pergamon Press; 1992. p. 480.
8. Eroschenko VP. diFiore's atlas of histology with functional correlations. 11th ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
9. Connell J, Chaseling J, Page M, Wright K. Tissue preservation in extreme temperatures for rapid response to military deaths. Forensic Sci Int Genet. 2018;36:86–94.
10. Caputo M, Bosio LA, Corach D. Long-term room temperature preservation of corpse soft tissue: an approach for tissue sample storage. Investig Genet. 2011;2:17.
11. Prinz M, Carracedo A, Mayr WR, Morling N, Parsons TJ, Sajantila A, et al. DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI). Forensic Sci Int Genet. 2007;1(1):3–12.
12. Green MR, Sambrook J. Isolation of high-molecular-weight DNA using organic solvents. Cold Spring Harb Protoc. 2017;2017(4):pdb.prot093450.
13. Rizky BN, Amiatun Ruth MS, Yudianto A. DNA purity and concentration analysis from toothpick as the evidence for forensic examination. Malaysian J Med Health Sci. 2021;17(Supp2):89–91.
14. Thermo Fisher Scientific. Quantifiler™ HP and Trio DNA Quantification Kits: user guide. Warrington: Thermo Fisher Scientific; 2018.
15. Williams G, Foley MM, Knight KL. Applied Biosystems' GlobalFiler™ PCR Amplification Kit. Methods Mol Biol. 2023;2685:241–52.
16. Tan P, Pepin É, Lavoie JL. Mouse adipose tissue collection and processing for RNA analysis. J Vis Exp. 2018;(131):57026.
17. Roy D, Tomo S, Modi A, Purohit P, Sharma P. Optimising total RNA quality and quantity by phenol-chloroform extraction method from human visceral adipose tissue: a standardisation study. MethodsX. 2020;7:101113.
18. Kochański AM, Mejnartowicz JP, Latos-Bieleńska A, Etienne J, Filipczyński L. DNA damage induced by lithotripter generated shock waves: short report. Int Urol Nephrol. 2001;32(3):419–22.
19. Rampant S. The effects of a detonation explosion on the recovery of DNA from fragments of an improvised explosive device [master's thesis]. Perth: Murdoch University; 2017.
20. Al-Snan NR. The recovery of touch DNA from RDX-C4 evidences. Int J Legal Med. 2021;135(2):393–7.
21. Berti A, Barni F, Virgili A, Colozza C, Maiorino F, Tocca M. The recovery of DNA profiles from saliva and touch evidences after postal bomb explosion. Forensic Sci Int Genet Suppl Ser. 2011;3(1):e471–2.
22. Hoffmann SG, Stallworth SE, Foran DR. Investigative studies into the recovery of DNA from improvised explosive device containers. J Forensic Sci. 2012;57(3):602–9.
23. Goodwin W, Linacre A, Hadi S. An introduction to forensic genetics. 2nd ed. Chichester: John Wiley & Sons; 2011. 198 p.
24. Ojo-Okunola A, Claassen-Weitz S, Mwaikono KS, Gardner-Lubbe S, Zar HJ, Nicol MP, et al. The influence of DNA extraction and lipid removal on human milk bacterial profiles. Methods Protoc. 2020;3(2):39.
25. Butler JM. Avanced topic in forensic DNA typing: interpretation. Oxford: Elsevier Academic Press; 2015.