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Case report

Experience based efficient approach for DNA-led identification of highly carbonized human remains

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CRediT authorship contribution statement

Denise Gianfreda: Conceptualization, Writing – original draft. **Beatrice Corradini**: Conceptualization, Writing – original draft. **Gianmarco Ferri**: Conceptualization, Methodology, Investigation, Writing – review & editing. **Francesca Ferrari**: Methodology, **Ilaria Borciani:** Methodology, **Rossana Cecchi**: Writing – review & editing, Supervision. **Anna Laura Santunione**: Supervision.

Abstract

In case of severely burned bodies, victim identification by visual or fingerprints recognition is often prevented by altered body conditions. To overcome these circumstances, different techniques are available. Among these, the most reliable is molecular identification, especially in cases of detached body parts. However, DNA analysis of highly burned remains is a very challenging task. The high temperatures reached at the time of burning can lead to the complete destruction of the genetic material, making DNA typing arduous.
This work presented a successful identification through molecular analysis of 11 heavily carbonized victims assigned to the Institute of Legal Medicine of Modena (Italy) between June 2022 and June 2023: a helicopter crash, a femicide and two car accidents.
Post-mortem (PM) and ante-mortem (AM) data were compared, allowing victims' identification and their quick return to relatives. Complete autosomal and Y chromosome STRs profiles were obtained for all the corpses. For the helicopter crash case, the utility of the DVI module implemented in the Familias software is shown as this aid the fast association of the seven victims involved with the familiar references available for identification.
The importance of the sampling strategy and the need of a systematic approach to select the most promising biological material for a more successful downstream DNA-based identification is also highlighted.

Keywords

Human identification, Femicide, Carbonized bodies, Disaster victim identification (DVI), Familias, Burnt remains, DNA profiling.

Highlights

- 11 heavily carbonized victims of helicopter crash, femicide and two car accidents were successfully identified through DNA analysis.

- The inner part, potentially less affected and damaged by high temperatures, of at least two types of soft muscle or organs tissues was sampled to increase full DNA typing.
- Comparisons of post-mortem (PM) and ante-mortem (AM) data were carried out.
- Familias software's DVI module was helpful for the small-scale disasters reported.
- Collaboration with Interpol was crucial for victims' efficient and fast identification.

1. Introduction

The identification of the victim is one of the first question requested by authorities when an unrecognizable or unknown deceased person is discovered [1]. When the identification through documents, fingerprint, visual recognition or surgical implants is prevented, a multidisciplinary approach is usually implemented and different branches of forensic medicine, like radiology, genetics, anthropology, odontology, are involved in this process.

Among the most complex forensic caseworks are those concerning fire victims, due to multiple alterations on the corpse caused by the flames [1,2]. Different techniques are available to overcome these challenging situations, the most reliable is to perform a molecular identification, which also allows to reassembly the detached body parts when needed.

In particular, forensic genetics should compare post-mortem (PM) and ante-mortem (AM) data for identification: PM data are retrieved from the victim body, instead of AM that are retrieved from the relatives or missing person's personal objects [3].

The importance of DNA analysis as fundamental tool in complex identification cases is widely endorsed, especially for those concerning mass disaster and missing persons [4-5]. However, DNA-based identification of highly burned remains is also a challenging matter, due to the high temperatures reached at the time of burning that can lead to the partial or complete degradation of the genetic material, which varies among tissue types [6].

Consequently, specific approaches should be taken to obtain a comparable genetic profile. Moreover, the forensic analyst must find alternative samples containing viable DNA sources when soft tissues are compromised.

Here four different DNA-led identification caseworks of extensively burnt human remains are shown, involving a total of eleven victims of a helicopter crash, a femicide and two car accidents assigned to the Institute of Legal Medicine of Modena (Italy) during the course of one year (June 2022 - June 2023). The body remains were discovered in a heavy state of carbonization, making impossible to recognize the physiognomic features as well as the reassembly of body parts. No useful elements for immediate identification like identity documents, personal effects or dental/surgical implants were found. Therefore, victims' identification was performed through DNA analysis. After the medico-legal surveys in the discovery area, all the remains were taken to the mortuary service of the Forensic Medicine division to perform autopsies and identification analyses. Among those presented, one case involved a body in extreme conditions, almost totally incinerated. The approach adopted for molecular identification and the challenges encountered in each casework are highlighted.

2. Case history

2.1. Case 1 - Helicopter crash

In June 2022, the traces of a helicopter belonging to a private company with six entrepreneurs on board plus the pilot, have been lost near the Tuscan-Emilian Apennines. After two days of searching, the remains of the chopped helicopter were found by a hiker in an area very far from the place of disappearance, presumably crashed due to the bad weather conditions encountered during the flight. The passenger's list was available, it was known that passengers were not related to each other and no people extraneous to the accident were involved. All seven passengers were found around the impact zone, the corpses were almost completely burned and some bodies 'detached parts were found on the ground. The victims were of three different nationalities. For this reason, for non-Italian victims, collaboration with Interpol was necessary. The degree of burning and degradation of the seven victims varied between the range of Level 3 (D3) and Level 4 (D4) according to the Crow-Glassman scale [7,8].

2.2. Case 2 - Femicide

In November 2022, the carbonized remains of a young woman were discovered in the trunk of her car close to a countryside area. The vehicle was probably set on fire by her offender, in the attempt to obstruct the investigation of the death. The car was powered by LPG fuel and, according to available circumstantial information, the gas combustion continued for about one hour reaching extremely high temperatures. In fact, LPG combustion can reach nearly up to 1925°C. Then, it is easy to deduce how exposing a body to these extremely degrading conditions for a long time leads to a high level of thermally induced tissue damage. On the corpse inspection, a severe and widespread charring process was evident. It involved the entire body showing the anatomical parts related to the upper and lower limbs almost completely calcined (**Figure 1**), with a total degree of burning classified between the Level 4 and 5 according to the Crow-Glassman scale [7], leading to an extent of body degradation of D4-D5 [8]. Based on the visual inspection of the body, there seemed to be no chance of success in obtaining a quality genetic profile usable for identification.

2.3. Case 3 - Car accident 1

In February 2023, the body of a woman was found near her car down a cliff, which caught fire following a runoff the road. Her corpse was found charred with a degree of burning and degradation classified between Level 3 (D3) and Level 4 (D4) on the Crow-Glassman scale [7,8].

2.4. Case 4 - Car accident 2

In June 2023, the carbonized bodies of a man and a woman were found inside their burnt car. The two people were victims of a traffic accident and probably their vehicle caught fire after a head-on collision. The corpse subsequently identified as the woman was the most charred, reaching a degree of burning and relative degradation tending to Level 4 (D4), while the male victim reached a burning degree of the Crow-Glassman scale tending to Level 3 (D3) [7,8].

3. Materials and methods

DNA-led identifications were performed by comparing PM and AM data according to international recommendations [9].
PM biological samples were collected from the victims' remains during autopsies.
With the aim of increase the chances of having full DNA profiles, assuming the possible DNA degradation and a reduced yield, different types of soft and hard tissues were collected for DNA analysis from each corpse when available, among which muscle tissues were preferred.
For muscle tissues or organs, it was chosen to cut and sample an area in the inner part as potentially less affected by high burning temperatures, avoiding the skin or the external part of the organ.
For identifying the victims, kinship or direct DNA identification approaches were both used. DNA profiles to be used as AM data were obtained from relatives' buccal swabs and from genetic data of family members provided by the Interpol system or from missing persons' personal items.
The type of AM and PM samples collected for each case are listed in **Table 1** and those chosen for DNA extractions are highlighted in bold.
For Case 1, a reference DNA profile from each of the seven victim's relatives was collected as AM data. The cooperation with the Interpol [10] allowed the availability of one close family member's DNA profile for each non-Italian victim, obtained with PowerPlex® Fusion 6C System (Promega), GlobalFiler Kit (Applied Biosystems) and Yfiler Plus Kit (Applied Biosystems) from the forensic laboratories of their respective countries. The DNA profiles of the Italian pilot's relatives were obtained through buccal swabs.
For Case 2, the collection of PM samples has been extremely challenging due to the severe body impairment. Several soft tissues were sampled in order to try to find some preserved areas.
Direct reference samples of the victim were collected and used as AM data.
Among the personal objects available, only two were finally used for DNA extraction (**Table 1**).
For Case 3 all the three PM samples collected during autopsy (psoas, quadriceps and liver tissues) were subjected to DNA extraction. As for Case 2, direct reference samples were collected as AM data. The following personal items were available: a toothbrush, a face sponge and contact lenses. Among those, DNA was extracted only from the toothbrush and from the face sponge.
For Case 4 soft autoptic tissues were collected as PM samples. For the first victim, tissues from the thighs were sampled, while for the second one, psoas and gluteus. As AM data for comparison, buccal swabs from one of each missing person's parent were collected (**Table 1**).
A portion of ~ 0.2-0.3 cm^2 of PM soft tissue, roughly chopped into small slices and placed in NAO® Basket (Copan Diagnostics) devices, was subjected to the DNA extraction procedure with QIAamp DNA Investigator Kit (Qiagen). The manufacturer's protocol for tissues was applied slightly modified, increasing lysis buffers as well as Proteinase K volumes, DNA was eluted in a final volume of 30 µl of ATE buffer.
At least two different types of PM tissue per victim were chosen evaluating those with a better state of preservation based on the visual post-mortem characteristics. For each type, DNA extraction was performed in duplicate.
DNA was extracted from personal objects using NAO® Basket (Copan Diagnostics) and QIAamp DNA Investigator Kit (Qiagen) dedicated protocols. Buccal swabs were extracted using ReadyAmp™ Genomic DNA Purification System (Promega).
DNA quantity was checked with NanoDrop 2000 (ThermoFisher Scientific). For Case 2, given the high level of damage of the charred remains and the limited availability of usable biological material also a qPCR analysis using the PowerQuant® System (Promega) on a 7500 Real-Time PCR System (Applied Biosystems) was conducted to evaluate the DNA availability and the level of degradation of PM samples.
Autosomal DNA profiles were obtained for all cases with PowerPlex® Fusion Kit (Promega) and for Case 1 also GlobalFiler™ IQC Kit (Applied Biosystem) and PowerPlex® Y23 Kit (Promega) were used in addition. PCR amplifications were carried out on a Veriti™ 96-Well Thermal Cycler (Applied Biosystems). Genotyping analyses were performed on a 3130 Genetic Analyzer (Applied Biosystems) sequencer coupled with GeneMapper ID-X v1.5 software.
DNA typing results interpretation and statistical evaluation were performed according to the Ge.F.I. recommendations for personal identification and parentage testing and a LR threshold of 10^6 was set [11]. For LR resulting between 10^2 and 10^6 analysis was extended to additional autosomal and Y-chromosome STRs markers.
The minimum number of loci to consider a profile for interpretation was set to 11 plus Amelogenin, as indicated in the validated criteria of the International Commission on Missing Persons (ICMP) [12].
Likelihood Ratio (LR) calculations for kinship analysis and direct match evaluations were performed with PatCan2 v1.0.11 software [13] and results were further confirmed with Familias v3.3.1 software [14]. For Case 1, although the size of the accident was contained, analyses were conducted following ISFG recommendations for Disaster Victim Identification (DVI) [9]. For this purpose, the DVI module of Familias implementing STRidER European frequencies [15] was used to pair PM data with AM data. The Direct Match Search option of the Blind Search function was used to assign the unidentified body parts (hand and foot) to the specific

victim. The YHRD database was used to analyze the Y-STRs profile of victim 6 [16]. In parallel, the pairwise search options were implemented to match victims and reference families, subsequently the established relationships were further verified through the traditional Kinship analysis tool. For the Victim 6, autosomal STRs genotyping analysis was also extended to Global Filer IQC Kit (Applied Biosystems) in order to confirm the value of LR obtained with Familias' Blind search and Y-STRs genotyping was also conducted.

4. Results

4.1. Case 1

Complete DNA profiles were obtained for all the seven victims as well as for the pilot parents' buccal swabs. The Familias software results are shown in **Figure 2-3.** A shared Y haplotype between the victim and the alleged brother allowed to provide a strong support to the identification hypothesis [3], strengthening the Familias software results. A conclusive identification was reached for all the seven victims involved.

4.2. Case 2

The extracted samples reported a Degradation Index (DI) of 14.08, 14.96 and 4.62 for Liver tissue 2, M. rectus femoris 1 - M. rectus femoris 2A respectively. All 24 STR markers amplified with PowerPlex® Fusion System (Promega) were successful typed for all PM data. Although M. rectus femoris 1 was the sample with the highest DI value, it was possible to obtain a complete profile from it, albeit with a marked characteristic 'ski-slope' pattern. DNA extracted from victim's personal items (Panties 1 and Panties 2) returned complete single profiles. Despite the extreme degradation condition of the body, a successful identification was reached a LR of 10^{27}.

4.3. Case 3

DNA extracted from the AM samples (toothbrush and face sponge) returned complete single profiles suitable for comparison. Statistical evaluation results in a LR of 10^{30} that provided extremely strong support for the hypothesis that the unidentified body belong to the missing person, rather than the alternative hypothesis.

4.4. Case 4

Identification was performed through parentage testing. The Blind Search function of Familias software was used to match relatives and victims' profiles, subsequently relationships were confirmed through traditional kinship analysis with PatCan and Familias software. Each victim-relative pair result in a LR of 10^{7}, providing an extremely strong support to identification.

5. Discussion

Successful DNA-based identification of heavily burned victims is a challenging task for forensic services due to the difficulty of recovering usable genetic profiles due to thermal exposure from such compromised material and at the same time the need to return the corpses to their families as soon as possible.

Recommendations [3,8,9,11], collaborative exercises [17-19] and real examples of mass fatalities [21,21] or missing person identification cases [5] are constantly reported in literature, especially after the great events occurred over the years. In fact, after the terrorist attack on the World Trade Centre and the South East Asian tsunami disasters [4,22], guidelines and international standards were better established to help forensic experts in the identification of the victims.

Of fundamental importance, especially in cases of profoundly degraded body remains exposed to fire, is the choice of the most suitable sources of biological material for DNA recovery. However, sometimes finding the right samples to use is very uncertain. A DNA-led identification can be challenging when heat and flames damages the conservation of the body, as DNA molecules could be affected by different levels of degradation and tissue-specific differences could be observed.

Senst et al. [8] published recommendations concerning the successful identification of altered human remains from compromised soft and hard tissues samples, including carbonized ones. Peripheral blood or other fluid samples are usually not the ideal choice in cases involving burned remains as they are reported to give the worst results in terms of DNA yield [8].

Blood samples analyzed in Case 1 returned the worst DNA profiles, showing high levels of degradation, and therefore no even sampled in the next three caseworks. According with the above cited work, the results obtained confirms useless of such samples, being the first to deteriorate.

Based on the suggestions reported in the ISFG and Interpol the DVI guidelines [2,9] also the sampling of bladder swabs is possible and it's corroborated by practical application [23,24], this procedure could also not be applied in the cases illustrated here as the bladder was no longer available.

The usability of the spinal cord as source of DNA in charred remains is also described [25]. However, this requires a more invasive sampling procedure than that used for soft muscle tissue collection, thus, this source cannot be considered as the primary choice.

Bones and teeth were also sampled in Case 1 victims, but not analysed because incinerated and would have hardly returned good DNA results. Either, as stated in Senst et al. [8], hard tissues such as bone samples are not the first selected when also soft ones are available, as they required a more complex and time-consuming preparation and processing.

Therefore, even in cases with an extensive burn destruction, it is advisable to try to find an area of the body where there is still a good quality tissue as by visual inspection, since a very small fragment is enough for genetic identification, as indicated in the materials and methods of this work. Small samples from the most central areas of the organs/tissues have most likely been preserved from the injuries of the flames, as demonstrated in **Figure 4.**

Nevertheless, despite having used for identification only the narrowest range of PM samples available for each victim in order to avoid further alteration of an already compromised body, it was possible to obtain successful DNA profiling for all the 11 victims.
In the case of uncertain LR value, where both the chances of false-positives and false negatives are high, the genetic typing should be extended, if possible, to additional STR loci and either Y-chromosome STRs or mtDNA variants as indicated in Bertoglio et al. [26].
Among the cases described here, Case 2 was the most challenging to treat due to the high carbonization degree of the body. It was almost completely characterized by an evident and widespread state of calcination, making the search for the biological material to be collected very long and difficult.
In cases like this, the advice is to try to find portions of tissues less affected by incineration (as per visual inspection less dark and closer to its natural colour) and to take samples of smaller portions to look for points where the DNA is potentially less degraded.
The use of appropriate software is essential for comparison purposes in mass casualty incidents or missing person identification cases, especially when the scale of the event is very large and the geneticist has to deal with a wide genetic profiles' database of PM and AM data. The utility and the applicability of the DVI module also to Case 1, even if it was not on a large-scale dimension, was demonstrated highlighting the importance of the availability of such versatile and highly efficient tool. In cases involving foreign persons, an effective collaboration with international organizations such as Interpol is very important for the forensic genetics laboratory in order to provide an efficient and faster identification of the victims.
The statistical calculations performed for family-based identification reflected the importance of using primary relatives for identifying victims as stated in literature [26]. Having more primary relatives available is expected to have an impact in victim identification success rate [27,28]. A systematic sampling approach for PM and AM documentation is crucial to select the most suitable biological material for DNA analysis. To date, the high sensitivity of the available modern forensic techniques allows to quickly obtain accurate genetic results even from severely compromised remains and, together with the accessibility of advanced statistical tools, the possibility to obtain reliable results by less labour intensive and less time consuming, significantly reducing the time to reunification.

6. Conclusion

DNA retrieval from thermally damaged tissues still continue to represent a challenging task for forensic genetics. Some important lessons learned from this experience are summarized below. First, the importance of sampling more than one tissue type among those available and prefer those with a less alteration state. Preference should be given to soft tissues because, even if they appear to be extensively affected by carbonization on the outside with hardening of the external part, it could be possible to find more preserved areas on their inside and therefore more suitable for obtaining a genetic profile. Of great importance, in cases such as those presented here, is the joint work of different forensic disciplines as well as the collaboration with international organizations. Finally, training of the laboratory personnel and the publication of specific case reports have great importance as each case has its own issues and literature helps the forensic scientists to choose the best way to carry out the analysis based on indications and suggestions given by experiences from other laboratories.

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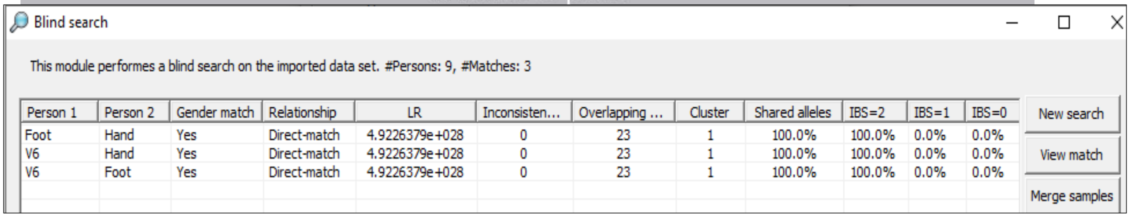
	AM data		PM data	
	Reference	**Source of DNA profile**	**Unidentified remains**	**Biological samples collected**
Case 1	Mother and Father (F1)	**Buccal swabs**	Victim 1 (V1)	**Psoas** - Sternum tissues - **Blood** - Teeth
	Father (F2)	INTERPOL	Victim 7 (V7)	**Muscle tissues 1** - **Muscle tissues 2** - Oral tissue - Mandibular - Teeth - Bones
	Father (F3)	INTERPOL	Victim 2 (V2)	**Muscle tissues 1 - Blood**
	Brother (F4)	INTERPOL	Victim 6 (V6)	**Muscle tissues - Liver tissues** - Bones
	Father (F5)	INTERPOL	Victim 3 (V3)	**Muscle tissues** - Mandibular - **Oral tissue** - Teeth
	Mother (F6)	INTERPOL	Victim 4 (V4)	**Muscle tissues - Blood**
	Father (F7)	INTERPOL	Victim 5 (V5)	**Muscle tissues 1** - **Muscle tissues 2** - Teeth
	/	/	Hand (H)	**Muscle Tissue - Nails**
	/	/	Foot (F)	**Muscle Tissue1 - Muscle Tissue2**
Case 2	Personal objects	Toothbrush 1 - Toothbrush 2 - Toothbrush 3 - **Panties 1 - Panties 2**	Female corpse	**M. rectus femoris 1 - M. rectus femoris 2A -** Femoral muscle 2B **- Liver tissue 1 -** Liver tissue 2 - Left leg muscles - Lung tissues - Brain tissues - Cerebellum tissues
Case 3	Personal objects	**Toothbrush - Face sponge** - Contact lenses	Female corpse	**Psoas - Quadriceps - Liver tissues**
Case 4	Father (F1)	**Buccal swab**	Victim 1 (V1)	**Left thigh tissues - Right thigh tissues**
	Mother (F2)	**Buccal swab**	Victim 2 (V2)	**Psoas - Gluteus**

Table 1: *Summary of AM and PM data collected for each case, in bold are highlighted samples subjected to DNA extraction procedures.*

Figure 1: *The almost calcinated remains of the Case 2 victim.*



Person 1	Person 2	Gender match	Relationship	LR	Inconsisten...	Overlapping ...	Cluster	Shared alleles	IBS=2	IBS=1	IBS=0
Foot	Hand	Yes	Direct-match	4.9226379e+028	0	23	1	100.0%	100.0%	0.0%	0.0%
V6	Hand	Yes	Direct-match	4.9226379e+028	0	23	1	100.0%	100.0%	0.0%	0.0%
V6	Foot	Yes	Direct-match	4.9226379e+028	0	23	1	100.0%	100.0%	0.0%	0.0%

Figure 2: *LR values of the re-association of body parts with Victim 6 (Case1).*

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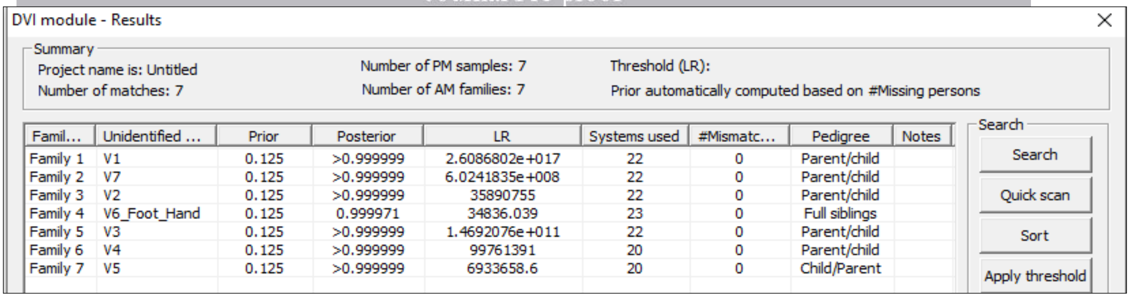
DVI module - Results

Summary
Project name is: Untitled
Number of matches: 7
Number of PM samples: 7
Number of AM families: 7
Threshold (LR):
Prior automatically computed based on #Missing persons

Famil...	Unidentified ...	Prior	Posterior	LR	Systems used	#Mismatc...	Pedigree	Notes
Family 1	V1	0.125	>0.999999	2.6086802e+017	22	0	Parent/child	
Family 2	V7	0.125	>0.999999	6.0241835e+008	22	0	Parent/child	
Family 3	V2	0.125	>0.999999	35890755	22	0	Parent/child	
Family 4	V6_Foot_Hand	0.125	0.999971	34836.039	23	0	Full siblings	
Family 5	V3	0.125	>0.999999	1.4692076e+011	22	0	Parent/child	
Family 6	V4	0.125	>0.999999	99761391	20	0	Parent/child	
Family 7	V5	0.125	>0.999999	6933658.6	20	0	Child/Parent	

Figure 3: *Posterior probabilities and LR values assigned to each victim and reference family pair (Case 1).*

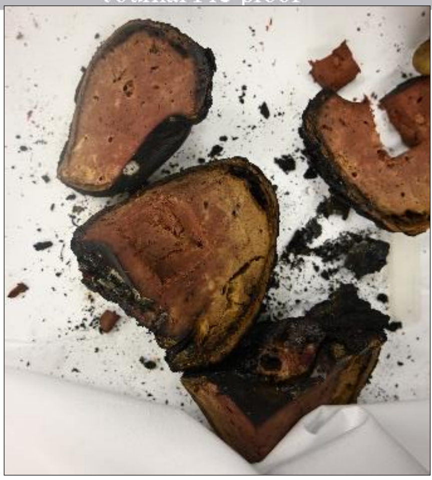

Figure 4: *Case 2 victim's liver tissue showing a more evident carbonization on the external surface.*

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- 11 heavily carbonized victims of helicopter crash, femicide and two car accidents were successfully identified through DNA analysis.
- The inner part, potentially less affected and damaged by high temperatures, of at least two types of soft muscle or organs tissues was sampled to increase full DNA typing.
- Comparisons of post-mortem (PM) and ante-mortem (AM) data were carried out.
- Familias software's DVI module was helpful for the small-scale disasters reported.
- Collaboration with Interpol was crucial for victims' efficient and fast identification.