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TECHNICAL NOTE

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Forensic Imaging-Guided Recovery of Nuclear DNA from the Spinal Cord*,†

ABSTRACT: Our objective is to document the recovery of DNA from the spinal cord or surrounding dura mater in 11 cases of severely burned human remains. Radiographs established that portions of charred tissue contained spine segments. Multidetector computed tomography (MDCT) revealed that each spine specimen contained an intact spinal cord remnant. A full DNA profile was obtained from seven specimens using spinal cord dura mater in six specimens and spinal cord medulla in one specimen. A partial profile was obtained from four specimens (spinal cord dura mater, 2; spinal cord medulla, 2). Bone and muscle surrounding the spinal cord appear to insulate nucleic acid containing tissue from critical thermal degradation. The spinal cord, which is easily identified by MDCT examination of remains and easily recovered at the postmortem examination, can be a source of DNA with extraction yields comparable with other tissue sources. Specimens of dura mater are preferable as processing time is faster than bone.

KEYWORDS: forensic science, forensic pathology, burns, computed tomography, nuclear DNA, spinal cord, forensic imaging, virtual autopsy

When badly burned bodies are found, positive identification is expected to be problematic. This is especially the case in mass disasters where there are large numbers of bodies and severe incineration has taken place. Strategies for identification include forensic radiology, forensic odontology, and recovery of DNA for testing. In practice, all methods are simultaneously used as no single method can be relied upon to be 100% successful (1,2).

The spinal cord was considered as a possible source for DNA sampling when virtual autopsy radiographic images showed intact portions of spinal cord in blocks of severely charred human remains. We describe our experience in 11 severe burn cases. Our hypothesis is that the spinal cord, when encased by bone and paraspinal muscle tissue, is protected from thermal degradation and will yield usable DNA for identification testing.

Methods and Materials

The protocol for processing severely charred tissue remains at the Office of the Armed Forces Medical Examiner (OAFME) was utilized in each case. It begins with assignment of case number; photography; identification (by simultaneous multidisciplinary methods); imaging studies; and, finally, complete autopsy with external and internal examination, generation of samples for toxicologic analysis, and diagrammatic documentation. Cases for which identification cannot be conclusively established with conventional methods are subjected to further study using anthropological methods and nucleic acid analysis (3).

As noted, all cases are radiographed. Radiographic imaging consisted of both digital radiographs (DR) and multidetector computed tomography (MDCT) of each charred block. The MDCT images were obtained on a GE Lightspeed 16 (General Electric [GE] Medical Systems, Milwaukee, WI) scanner at 0.625-mm contiguous intervals. The images were reconstructed as 1.25 mm slices and viewed on a GE Advantage Workstation (software Version 4.2; GE Medical Systems, Milwaukee, WI) using two-dimensional (multiplanar) and three-dimensional data sets.

According to the standard autopsy protocol, skeletal muscle is harvested for DNA analysis. In addition, for the 11 cases discussed, the standard tissue sample was supplemented using blood and bone, as well as a segment harvested from the spinal cord. Radiographic guidance was used to expose the spinal cord using standard techniques (4,5), and the cord tissue obtained was submitted for possible DNA recovery.

DNA processing of the spinal cord samples submitted involved the extraction of nuclear DNA from the spinal cord for short tandem repeat (STR) analysis to compare against reference samples. Approximately 2-inch long segments of the spinal cord (Fig. 1) were submitted for DNA processing. A 2-mm square portion of the spinal cord dura mater or medulla was obtained for DNA chelex extraction in accordance with the Armed Forces DNA Identification Laboratory (AFDIL) standard operating procedure for tissue extraction (6). The extract was then quantified to determine the amount of DNA present in the extract. Based on the amount present, the sample was amplified using Quantifiler Human DNA Quantification Kit (Applied Biosystems, Foster City, CA) targeting the optimum input volume using PowerPlex 16 Amplification Kit (Promega, Madison, WI) for polymerase chain reaction (PCR). The PCR product was then subjected to capillary electrophoresis on the

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FIG. 1—*Spinal cord specimens used for DNA extraction. Upper lumbar cord sample from which dura mater yielded a full DNA profile.*

ABI Prism 3130xl genetic analyzer (Applied Biosystems, Foster City, CA) to generate the DNA profile.

Results

The 11 cases were remains resulting from explosions with ensuing fires sustained by ruptured (vehicular) gasoline tanks. The size and condition of the remains varied from charred fragments to charred torso remnants with thermal amputation of head and extremities. Five cases were torso remains, four were cervical and thoracic blocks, and two were lumbosacral blocks (Table 1). In all cases, assessment of mechanism of injury was compromised by the thermal changes and absence of suitable samples for determining systemic circulating levels of products of combustion. In such instances, more information may be derived from aggregated results if remains belonging to distinct individuals can be reliably discriminated and separated. Although the samples processed were combined from separate incidents, they are similar to sets of remains obtained from single episodes involving multiple individuals both in terms of size of sample usually encountered and in the range of marked thermal effects usually seen.

The radiographs and MDCT survey of each case established the presence of the spine. For example, Case 1 is a block of charred tissue sent for identification. Case 1 was a lumbar spine segment with attached liver. It extended from T10 through the sacrum (Fig. 2) and, within the canal, a portion of spinal cord with normal dimensions and a smooth surface (Fig. 3).

In each case, MDCT imaging allowed the reconstruction of images through the spinal canal in sagittal and coronal planes. Typically, the spinal cord was outlined by air in the canal allowing assessment of continuity, thickness, and surface regularity. As illustrated by Case 1, the multiplanar images guided the pathologist to a location deemed appropriate for obtaining a cord sample. Nuclear DNA was recovered from the cord specimen and from bone in Case 1.

The DNA recovery results for the paired spinal cord samples and matched routine specimen samples submitted are listed in Table 1. In eight of the 11 cases, dura mater was the tissue source, and six of these samples yielded a full DNA profile. In the remaining three cases, medullary spinal cord was the tissue source, and of these, one case yielded a full DNA profile and two yielded a partial profile. Such parallel matched tissue samples are usually submitted but could be sent in only eight of the 11 cases due to extensive thermal degradation of the usual sample sources. These were from bone (1 site), tissue (3 sites), blood clot (3 sites), and lung (1 site), and all produced a full DNA profile. In the remaining three cases, only dura mater was left as a potential DNA source; in two of these three cases, a full profile was derived, while in the remaining case, a partial profile was obtained.

Discussion

Recovery of DNA from burned remains is dependent upon an adequate sample for extraction and subsequent amplification. Usual DNA sources become reduced as thermal effects increase due to prolonged burning at high temperature, resulting in incineration of head, extremities, ribs, and associated exposed soft tissue (7). Charred remains in these cases often consist of remnants of the axial skeleton such as pelvis and spine. Paraspinal muscle provides one source for sampling, and bone marrow is another. If usual sources of nuclear DNA are not usable because of thermal degradation, then noncharred bone can be used as a source of DNA (8). In this study, we show spinal cord and surrounding tissues to be comparable with other organ tissue as a source of nuclear DNA in severely charred remains. Processing time for spinal cord tissue is also faster than for bone.

In general, tissue samples require shorter laboratory processing time than bone samples. When an urgent sample is submitted to the laboratory for STR DNA results, tissue samples can require 24 h to process, whereas bone samples usually require 48 h. Spinal cord samples are processed as tissue samples for DNA extraction, therefore requiring less DNA extraction time and yielding faster results when compared with processing of bone samples.

TABLE 1—*Description of remains.*

Case	Anatomic Tissue from Which Sample was Taken	Spinal Cord Sampling	DNA Results*	Routine Sample	DNA Results*
1	Lumbosacral fragment	Dura mater	Full profile	Bone	Full profile
2	Thoracic fragment	Dura mater	Full profile	Lung	Full profile
3	Lumbosacral and partial pelvic fragment	Medulla	Partial profile	Spinal clot	Full profile
4	Torso fragment	Medulla	Partial profile	Clot	Full profile
5	Cervical thoracic fragments	Medulla	Full profile	Tissue	Full profile
6	Cervical and thoracolumbar fragment	Dura mater	Partial profile	Clot	Full profile
7	Torso fragment	Dura mater	Full profile	NA	NA
8	Torso fragment	Dura mater	Full profile	NA	NA
9	Torso fragment	Dura mater	Full profile	Tissue	Full profile
10	Cervical upper thoracic fragment	Dura mater	Full profile	Tissue	Full profile
11	Torso fragment	Dura mater	Partial profile	NA	NA

*Full profile = 16 loci, partial profile = <16 loci.

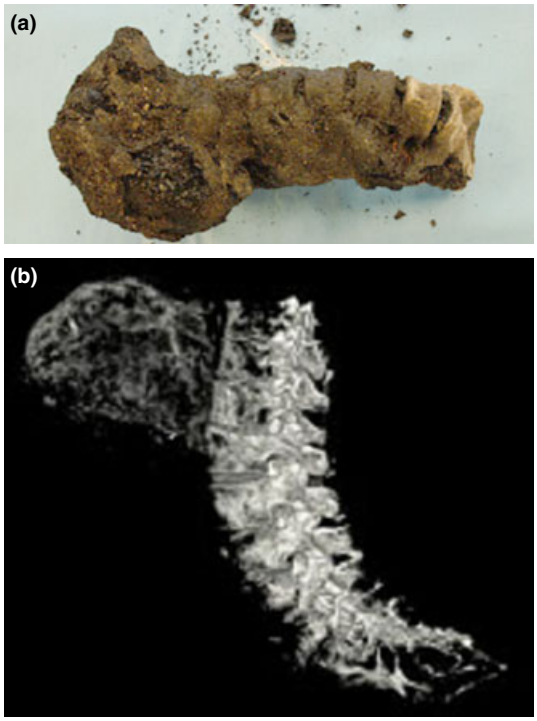

FIG. 2—*(a) Charred specimen of lumbar spine (Case 1). (b) Three-dimensional radiographic reconstruction of the specimen made with bone algorithm.*

It was noted in our study that the portion of the spinal cord sampled affected the success in obtaining a full DNA profile. Dura mater (outer layer) yielded better results than the spinal cord medulla (inner layer). The spinal cord medulla samples produced the best profile when a diluted sample of the extract was used for PCR amplification, indicating that some type of PCR inhibitor is present in the spinal cord medulla extract. The medulla showed inhibition during PCR, resulting in partial STR profiles. Our success with dura mater is consistent with the experience of Matani et al., who recovered DNA from calvarial dura mater in cadavers (9).

Neural tissue, while known to be a good source for nuclear DNA, is susceptible to degradation (8). Our cases reflect prolonged exposures to very high temperatures as indicated by the thermal amputation of all extremities and associated tissues except core tissue of the trunk. It is our belief that the muscle tissue and bone surrounding the spinal column protect the spinal cord from degradation. Location of spinal cord sample selection within the tissue block can influence the success of recovery. In Case 3, radiographs indicated the tissue contained spine from T-11 through the sacrum with fragments of right iliac and hip joint. The CT reconstructions showed cord intact at the conus medularis (L-2 level). Tissue samples of cord and blood clot in spinal canal and bone all yielded nuclear DNA; however, some degradation in this cord sample was noted. Because the distance from conus to exposed canal at T-11 is short, the sample site may be less "protected."

Radiology has a long tradition of aiding identification of burn victims by demonstrating skeletal characteristics unique to the premortem radiographs of an individual (10). In this report, we emphasize the use of new postmortem imaging techniques to assist the forensic pathologist prior to physical autopsy. Expansion of forensic radiology to include cross-sectional methods such as MDCT and magnetic resonance imaging (MRI) allows multiplanar two- and three-dimensional postmortem viewing of human remains and has been referred to as CT-assisted autopsy or "virtual autopsy" (11–13). The advantages of cross-sectional imaging include precision for localization and improved soft-tissue discrimination. A role for mobile computed tomography has been suggested in mass fatality incidents (14,15). In our series, the presence and physical status of the spinal cord was determined and guided the dissection to the area for sampling. The scan images cannot be used to judge microscopic changes, so presence of an intact cord does not ensure absence of degradation. In this series, a spinal cord segment with normal dimensions, smooth margins, and a lack of shrinkage implied no defects. Loss of volume is one of the hallmarks of organs such as lung, liver, and kidney when exposed to high heat.

This study is limited to spinal cord samples from the lower thoracic and upper lumbar spine, so it cannot be assumed that samples from other areas such as the cervical cord can be used in burn cases. The protective effect of the lower trunk is likely to be better than in the upper thorax and neck. When a long portion of the cord is present, it would seem prudent to obtain a sample in the lumbar or lower thoracic region. Also, local areas where thermal tissue loss is more severe or where trauma is present should be avoided when alternative sites exist. In this small number of samples, we did not show that cord tissue was better than the standard sites, such as blood. However, we demonstrate that cord specimens, in particular dura mater, provided acceptable samples for DNA extraction. The

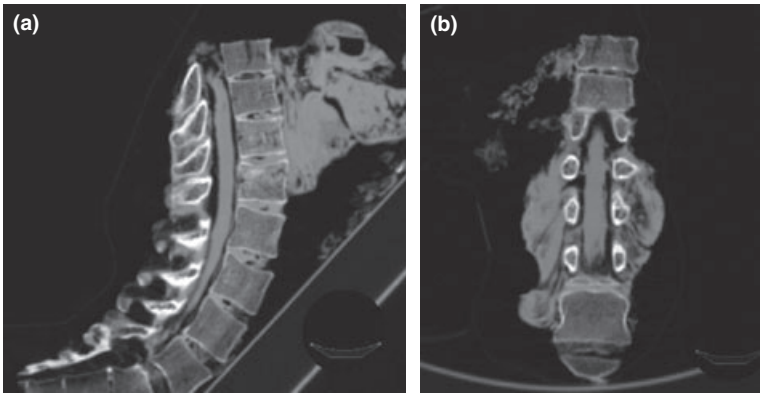

FIG. 3—*Two-dimensional reconstructed sections of Case 1 in (a) sagittal and (b) coronal planes through the spinal canal show the cord surrounded by air. In each section, the cord is intact, of uniform thickness, smoothly marginated, and shows homogeneous density. Note remnant of liver anterior to the upper spine in (a).*

ease in which cord specimens can be processed when compared with bone is an advantage. We expect that the combination of imaging techniques and harvesting of cord-associated materials (including bone obtained from the same site) will be valuable in generating identification matrices when identification is challenging because of thermal changes.

Conclusion

The spinal cord can be an accessible source of DNA that is comparable with tissue or organ samples when severe thermal effects produce charred remains of the lower torso. Processing time is faster than bone. Radiographic imaging with CT can guide the forensic pathologist to appropriate sites.

References

1. Valenzuela A, Marques T, Exposito N, Martín-De Las Heras S, García G. Comparative study of efficiency of dental methods for identification of burn victims in two bus accidents in Spain. Am J Forensic Med Pathol 2002;23:390–3.
2. Meyer HJ. The Kaprun cable car fire disaster—aspects of forensic organisation following a mass fatality with 155 victims. Forensic Sci Int 2003;138:1–7.
3. Gaenissien R, Weeden V. Forensic serology and DNA. In: Fierro M, editor. CAP handbook for postmortem identification of unidentified remains, 2nd ed. Northfield: College of American Pathologists, 1998;148–62.
4. Giannini C, Okazaki H. Nervous system. In: Ludwig J, editor. Handbook of autopsy practice, 3rd ed. Totowa: Humana Press, 2002;65–84.
5. Dolinak D, Matshes E. Medicolegal neuropathology. Boca Raton: CRC Press, 2002.
6. Armed Forces DNA Identification Laboratory. Chelex extraction of DNA from tissue and fresh skeletal remains and bode buccal DNA collectors. AFDIL DNA Extraction Manual Version 5. Washington, DC: Department of Defense DNA Registry, Armed Forces Institute of Pathology, manual adopted May 10, 1997.
7. Bohnert M, Rost T, Pollak S. The degree of destruction of human bodies in relation to the duration of the fire. Forensic Sci Int 1998;95:11–21.
8. Butler JM. Forensic DNA typing, 2nd ed. Burlington: Elsevier Academic Press, 2005.
9. Motani H, Sakurada K, Akutsu T, Ikegaya H, Hayakawa M, Sato Y, et al. Usefulness of dura mater in providing DNA samples for identifying cadavers. J Forensic Sci 2006;51:888–92.
10. Singleton AC. The roentgenological identification of victims of the ''Noronic'' disaster. Am J Roentgenol 1951;66:375–84.
11. Thali MJ, Yen K, Schweitzer W, Vock P, Boesch C, Ozdoba C, et al. Virtopsy, a new imaging horizon in forensic pathology: virtual autopsy by postmortem multislice computed tomography (MSCT) and magnetic resonance imaging (MRI)—a feasibility study. J Forensic Sci 2003;48:386–403.
12. Levy AD, Abbott RM, Mallak CT, Getz JM, Harcke HT, Champion HR, et al. Virtual autopsy: preliminary experience in high velocity gunshot wound victims. Radiology 2006;240:522–8.
13. Harcke HT, Levy AL, Abbott RM, Mallak CT, Getz JM, Champion HR, et al. Autopsy radiography: digital radiographs (DR) vs multidetector CT (MDCT) in high velocity gunshot wound victims. Am J For Med Pathol 2007;28:13–9.
14. Rutty GN, Robinson CE, BouHaidar R, Jeffery AJ, Morgan B. The role of mobile computed tomography in mass fatality incidents. J Forensic Sci 2007;52:1343–9.
15. Levy AD, Harcke HT, Getz JM, Mallak CT, Caruso JL, Pearse L, et al. Virtual autopsy: two- and three-dimensional multidetector CT findings in drowning with autopsy comparison. Radiology 2007;243:862–8.

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