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DNA REVIEW

DNA reviews: DNA identification following CBRN incidents

Eleni Socratous · Eleanor A. M. Graham

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Abstract Chemical, biological, radioactive, or nuclear (CBRN) incidents can occur due to accident or deliberate action, and may result in substantial loss of life. Whatever the cause, the requirement for identification of the deceased may necessitate the removal of contaminated samples to a DNA laboratory for processing. This review looks at the potential types of CBRN that may result in the requirement for DNA identification of the deceased and investigates the potential risks and difficulties associated with processing samples of this type.

Keywords Forensic science · DNA · Chemical · Biological · Radioactive · Nuclear · Identification

Introduction

Throughout the world, following the death of individuals despite the cause, there is a humanitarian and often legal requirement to ascertain the identity of the deceased. Positive identification can aid the grieving process of loved ones and, depending on the situation, assist investigations into the circumstances of the death. As previously reviewed [1], one of the four primary criteria, fingerprints, dental comparison, unique prostheses, or DNA profile, is sought to confirm the identity of the deceased. Each of these methods requires the manual inspection of the remains and especially in the case of DNA, may require the removal of samples for further specialized processing or inspection. Personal identification can present many challenges to the investigating team, especially following a mass fatality incident when large numbers of intact or fragmented bodies may require attention. These challenges are made even greater if the scene and remains have become contaminated with chemical, biological, radioactive, or nuclear (CBRN) material during the lethal incident. Contamination of the scene and remains may be the result of any number of situations including industrial accidents such as the nuclear disasters at Three Mile Island, Pennsylvania, in 1979 and the Chernobyl incident in 1986. Although fortunately no one was directly killed during these incidents, it is believed that many have since died as a result of the exposure to radiation causing various forms of cancer, most notably leukemia, and with the continued reliance on nuclear power, the potential for further incidents remains. In addition to accidental incidents, the threat of deliberate use of CBRN agents in terrorist attacks is a significant cause for concern in the world today.

CBRN warfare agents

CBRN terrorism is not a new concept. Biological agents were reportedly used in the first century B.C. when the Assyrians contaminated the water supplies of their enemies with hallucinogenic fungi [2]. In more recent history, the use of chemical warfare was well documented during World War II whereby vesicant sulfur mustard [3–5] and the choking agents phosgene and chlorine were used to kill almost 1.3 million people [3]. In the time following the end of the War, CBRN incidents have been reported sporadically, without public reaction on a large scale. It was the Japanese sarin nerve gas attacks at Matsumoto in June of 1994 and Tokyo subway attack in March of 1995 [6, 7] that

E. Socratous · E. A. M. Graham (✉)
East Midlands Forensic Pathology Unit, University of Leicester, Robert Kilpatrick Building, Leicester Royal Infirmary, Leicester LE2 7LX, UK
e-mail: eamg1@le.ac.uk

awakened the public to the potential devastation that could be caused by acts of CBRN terrorism.

Chemical warfare agents are substances with toxic properties, which are used in an attempt to kill, injure, or incapacitate people. They are categorized based on the effect they have on humans [5, 8]. They are usually classified into five groups: choking/pulmonary agents, incapacitating agents, biotoxins, vesicants such as sulfur mustard and lewisite, and nerve agents. The most lethal group is nerve agents, which includes substances such as sarin and tabun. Nerve agents can be extremely toxic causing blocking of the enzyme acetylcholinesterase,which results in the contraction of muscles and continuous stimulation of the nervous system which can lead to coma and death [9, 10]. Biological warfare agents are pathogens or biotoxins that can cause life-threatening illness to humans, animals, or plants [11]. This type of warfare is perhaps the most feared since they are odorless and tasteless, and can stay undetected for hours, days or until the victims of the attack show symptoms of the illness [11, 12]. These agents are classified into three groups according to the mortality rates upon exposure. Category A includes the high threat agents such as anthrax or smallpox which can be fatal even in small doses. Categories B and C comprise threats that have low mortality rates and causing diseases that could be potential threats in the future, respectively [10].

A radiological attack is defined as the dispersal of radioactive material with the intent to do harm. Small amount of radiological material can be found in our everyday life, in industry, laboratories, and several medical centers, making them accessible to potential terrorists. There are two types of radiation. The first type is non-ionizing radiation, which is not particularly damaging, and it is used in microwave ovens, toasters, television sets, etc. The only form of non-ionizing radiation that can damage tissue is UV-light, which has not to date been reportedly used as a weapon. The second type, ionizing radiation, is categorized into four types—gamma rays, X-rays, alpha, and beta radiation—according to the type of radiation emitted. This type of radiation can cause structural changes to DNA and tissues with potentially lethal effect [13]. It is considered that the greatest threat posed by the use of radioactive material is the creation of radiological dispersal devices (RDD) or “dirty bombs.” Unlike nuclear bombs whose explosion can affect large areas through fallout of the radioactive dust, a dirty bomb has more localized effects, as conventional explosives are used during dispersal and may only be capable of distributing the radioactive particles within a city block or a few square miles. The effects of a RDD explosion would result from the initial explosion, followed by radioactive dust in the bomb. The attack will immediately affect people in close proximity to the site of the explosion, but depending on the amount of radiation and the time exposed to it, the exposure effects can vary [9].

Nuclear weapons were developed during the 1930s and were designed to give the Allied forces the ultimate advantage during World War II. Upon detonation, nuclear weapons cause massive explosions due to the energy created during nuclear fission within the bomb assembly. This reaction first produces extreme blast energy followed by ionizing radiation, light energy, and heat energy. The results of a nuclear attack can be catastrophic resulting in considerable fatalities, injuries, and infrastructure damage. Moreover, the electromagnetic pulse from the explosion can kill by disrupting the signal of electronic devices and there can be late effects from the radioactive fallout. Nuclear explosions are categorized in relation to the amount of energy they produce. A terrorist nuclear attack is expected to produce energy of several kilotons of TNT, whereas the military nuclear weapons yield in megatons [5, 9]. Nuclear weaponry has twice been used on human populations, both on Japanese soil and both with devastating effect. At 08:15 local time on August 6, 1945, the first bomb was dropped and detonated over the city of Hiroshima, causing the instantaneous death of approximately 70,000 individuals with a further 70,000 subsequently dying as a direct result of this attack. Before the full effect of this attack had been registered by the global community, a second bomb was dropped at 11:02 on August 9, 1945, over the city of Nagasaki, killing another 80,000 individuals within a year of its use. Despite numerous calls for the destruction of all nuclear weaponry, many countries continue to produce, test, and stock weapons of this kind, maintaining the threat of their use on humankind.

Recovery and handling of contaminated samples

Despite the cause of the incident, be it accidental or deliberate, the contamination of bodies and scenes with CBRN agents will present a potential risk to all emergency workers that are required to assist with the recovery of the injured and deceased, make areas structurally safe, and investigate the cause of the incident. During the production of contingency plans designed to minimize the risk posed to those working at such scenes, one area in particular has not been fully investigated for its associated potential risks, DNA profiling for personal identification of the deceased. Currently, DNA profiling requires the removal of biological material from the remains to a laboratory at a fixed location, although the use of mobile laboratories is becoming a viable option for use in such situations. Wherever the laboratory is located, the effect of CBRN agents on the chemicals used in DNA profile production

and the potential of personnel exposure to hazardous materials during processing have not been fully investigated.

Recently a study was undertaken to assess nine chemicals that can potentially be used as weapons. This work investigated the effect of scene and laboratory decontamination techniques and also the chemicals themselves for effects on DNA profile production. During this work it was found that bleach-based decontaminating agents used at the scene or laboratory would adversely affect the chance of DNA profile production and should therefore be avoided on samples collected for use in DNA identification. Sodium hypochlorite, the main chemical ingredient of bleaches, is found to degrade DNA through oxidative damage. As a result, good quality DNA cannot be recovered from the samples and therefore full DNA profiles are not obtained [14]. Additional experiments were performed to deduce whether chemical contaminants can interfere with the production of a DNA profile. Nine chemicals were chosen for inclusion in this study based on their availability and likelihood of being used as weapons. The crude effect of these chemicals on DNA profiling reactions was tested by spiking blood with small quantities of each before processing using standard methods. Full DNA profiles were consistently obtained when conmatinated with hydrogen cyanide, sarin, sodium fluoroacetate, sulfur mustard, and diazinon. Partial DNA profiles were obtained after contaminating the blood samples with lewisite I, and no DNA profile was produced after additions of chlorine, phosgene, and dimethyl phosphate [14]. Additional testing was performed to assess the persistence of four of the tested chemicals in the sample, during DNA extraction, by using chemical analysis methods based on mass spectrometry and magnetic resonance technologies. It was found that sulfur mustard was rapidly degraded by incubation in DNA lysis buffer and sarin was removed during the washing steps rendering the elution buffer safe to handle for both chemicals after minor processing. On the other hand, traces of diazinon and sodium 2-fluoroacetate were detected after completion of the DNA extraction process but at relatively low levels compared to that entered into each spiked sample, indicating that extracted DNA would be safe to handle following normal extraction procedures [15].

Aside from the work discussed above, no other attempts to determine the effect of CBRN agents on DNA profile production have been published in the literature. For biological agents, it can be hypothesized that bacteria and viruses will not interfere with the production of a DNA profile, even if the final extraction product contains DNA from the agent used in the attack. This is mainly because DNA profiling kits are designed to be human specific and will not amplify the DNA of other organisms present in the template DNA. This can be illustrated using the example of decomposed cadaver, whereby large volumes of bacteria may be collected and co-extracted in samples collected for the purpose of personal identification. These bacteria are co-processed and the DNA of the bacteria will be present in the extracted DNA product. Full human-specific DNA profiles are still produced routinely from samples of this type, with little, if any, interference noted. This observation is only valid if the biological agent used is actually broken down and digested during the processing of the collected sample. This may not be the case for notoriously long-lived spores such as anthrax, which has been used in several terror campaigns in recent times [16–18] and which, due to their tough exterior, may remain unchanged throughout the DNA extraction process, and therefore remain a potential threat to persons handling samples of this type.

The use of ionizing radiation is common to nuclear and radiological attacks; the size and scale of the dispersal method however differ dramatically. As a result of any explosive event the potential for body disruption will add complication to identification efforts; this, coupled with the intense heat and blast associated with nuclear attacks, may render identification of all victims impossible due to the level of disruption and cremation observed. The handling of samples contaminated with sources of ionizing radiation has not been investigated for the laboratory environment. It is known that ionizing radiation can cause damage to the structures inside the cell including DNA [19]. The effect of radiation on those surviving the initial explosive event may result in mutation of the DNA and could lead to any number of pathological complications, most notably cancers [19–21], which as previously reviewed can have adverse effects on DNA profiling [22]. The effect on laboratory staff processing radioactively contaminated samples may be similar; no data has however been produced to assess this risk to date.

Summary

In recent times, mainly as a response to the continued threat of terrorist attacks, more attention is being paid to the preparation of CBRN response teams. These teams will be trained to enter the scene of contaminated incidents to recover the living and process the dead. Current practice has produced guidelines for the safe operation within contaminated scenes and the surrounding areas including decontamination and/or disposal of all clothing and equipment that has been inside the designated “dirty zones” following CBRN incidents [9, 13]. Very little has been published at the time of writing regarding the potential risks to personnel and methodological problems that may be associated with the processing of CBRN contaminated samples in the DNA laboratory. The article

by Wilkinson et al. [14] has provided us with an interesting insight into the use of decontamination techniques and the direct effects of potential chemical warfare agents including the observations that DNA samples should be collected before decontaminating the scene, especially if bleach-based agents are to be used. It would be interesting to assess the use of other decontaminating agents on DNA profile production as a method of avoiding any potential risks associated with handling of collected samples within the DNA laboratory. Furthermore, it would also be of great interest to perform studies investigating the effect of CBRN agents on more biological samples such as buccal cells, soft tissue, bone, and teeth, all of which may be recovered to aid personal identification of the deceased [14].

Educational message

(1) CBRN incidents may be the result of any number of events including industrial accidents and terrorist attacks.

(2) Following a CBRN incident great care must be taken to avoid spreading of contamination outside of the restricted "dirty zone".

(3) Processing contaminated samples after a CBRN attack can be challenging and dangerous. All samples should be handled with great care, under controlled conditions.

(4) Further research is required to determine the persistence of CBRN agents in biological samples collected for DVI.

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