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Personal Identification from Charred Bones

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Abstract

The charred bones are found in various cases like arson, explosion, fire, automobile accidents, plane crashing, etc. The charred bones change after burning at various temperatures and these changes can be studied to make observations. The changes are coloration change, reduction in weight, shrinkage and deformation, and fragmentation and survival of trauma. These changes various with the degree of burning i.e. the temperature of burning and are occurred accordingly. In severe burning, the organic matrix degrades and as well as the DNA. So, it is tough to get the DNA and perform the further analysis but there are some chances if there is any sort of recovery of DNA but then there is no guarantee for the results.

Keywords: Personal; Identification; Trauma

Introduction

Fire is amongst the destructive forces, which is capable of huge damage. Charred bones can be found in various situations like arson cases, accidental fires, vehicle accidents, bomb blasts, airplane crashes, volcanic eruptions, and modern cremations. The body of human has soft as well as hard tissues, fire have effects on both. The soft tissues contract leading to skin tear and further shrinking of muscles and fat. Teeth and bones are the last to get burned and the properties of bones change drastically both physically and chemically which hinders the forensic identification tests. Bone is composed of calcium phosphate and collagen which makes it strong and flexible so that it can. withstand stress. The skeleton does not burn in a uniform way. Some bones burn at greater intensity than the others due to various factors such as body fat distribution, closeness to heat, etc. Body fat play role as a fuel in burning of the body so, the more the fat an individual is, the more the intensity of burning will be.

Changes in Charred Bones

Coloration

When a bone is burnt, it changes its color drastically. The color change of the surface of bone varies with the exposed temperature and many researchers have tried to correlate the color change of bone with the temperature to which it is exposed. The degree of the color change of the charred bone also depends on the duration for which it is burning. The more the duration of burning, the more will be the severe color change. The coloration process is delayed by the anaerobic burning conditions. At the commencement of combustion, the soft tissues act as physical barrier against fire in a dead body. The thickness of the soft tissue is uneven and even in the one person there are different degrees of burn due to the different heat distribution during burning. The relation between bone color change and temperature is as follows.

Weight reduction

Combustion (burning) of organic materials and vaporization of water leads to the CO2 (carbon dioxide) release which results in bone weight reduction. The average cremated bones obtained after complete cremation is 2000g and 3000g for female and male respectively. The decrease in weight is extreme till 400°C, it inclines towards a plateau at probably 700°C, and stabilizes at around 60% of the weight that was originally.

Compact bone comprises of 14% H_2O, 24% organic matrix and 62% bone mineral, burning would not release it. Therefore, it is okay to say that water is lost by charred bone and a notable quantity of organic matrix is lost by 400°C and further it is completely lost by 700°C. There is also a major loss of collagen between 200°C and 400°C temperatures, turning lenient towards 700°C, at this the collagen is undetectable.

Shrinkage and deformation

There is also a volume reduction upon burning of bones. There is no quantitative analysis found till now due to the difficulty in

obtaining measurement precisely of its volume in the fragmented and cracked form. The volume measurement (digitally) of complex shapes are possible by the X – ray computed tomography has been used in volume analysis of charred bones. The correct measurements of volume reduction of burnt and cubically cut compact bones are obtained by micro – CT. There is zero volume change until 600°C but after that decrease considerably up to 1100°C by which the volume is almost half of what it was initially. The amalgam of collagen losing, recrystallization of hydroxyapatite (crystallinity increases), chemical change of the hydroxyapatite into beta – tricalcium phosphate and joining of these crystals causes shrinkage. There is a problem caused in anthropometric tests due to this shrinkage and deformation. Even after the weight reduction has come to an end, the shrinkage continues, and the compact bone density increase at high temperature (i.e. > 500°C) leading to bone hardening. Fredericks et al measured hardness of charred bone using Vickers hardness method. The results implied that the compact bone become brittle after a starting little hardening developed below 150°C, and when combustion depleted collagen structures, then stiffening and hardening initiated at 400°C. Above 700°C, there is an accelerated hardening with growing crystallinity measured by FTIR, as a splitting factor. During the action of this recrystallization, a diversity of distinctly sized and shaped crystals are observed using SEM at high magnification. There is a huge shape alteration noticed as hexagonal, platelets spherical rosettes occur at above 600°C in the crystals' growth. The temperature exposure and age of the person are the factors on which the making of these distinctive or unique crystals depends. There are cracks produced in the charred bones due to shrinkage, at above 500°C the minute cracks arising from Haversian canal can be observed. At higher temperatures, these cracks increase in sizes and numbers. After 1100°C, the cracking can also be seen using SEM as multiple fissures.

Fragmentation and trauma survival

Fragmentation is triggered upon origination of cracks in the material that is hardened. The charred bones possess fragments at distinct degrees this further poses difficulty in the identification process. Reconstruction is frequently applied on fragments of bone in identification process. If there is delay in recovering, then the fragmentation is increased. Freezing, temperature change and wet conditions are responsible for enhancing the fragmentation of charred bones. In homicide cases, the necessity is to specify the evidence of any trauma on charred bone and aim to depict the weapon utilized.

DNA Survival in Charred Bones

DNA analysis techniques are often applied for skeletal remains' identification after its progresses. Due to the fragmentation as well as deformation of charred bones, the morphological tests are difficult to perform and in such cases DNA profiling is used for identifying such severely burnt bones. But the harsh reality is that in early phase of burning itself the organic matrix disappears and so is the DNA without exception. Amplification of 120bp products of the human mt DNA were assessed by Cattaneo et al in experimentally burnt bones of human at 800°C - 1200°C for about 20 minutes in addition to charred bones gathered from real cases. They observed that in none of the bone's DNA was retained that was amplifiable and thus deduced that DNA typing is not capable of being used with charred bones successfully. However, results obtained by Schwark et al from amplifications of DNA (charred bones) were better. The degree of burn was categorized in accordance with the color of bone and they successfully gained elaboration from the specimens of "blue – gray – white" color due to highest degree of burning. The successful amplification appears to be against the results got from various other studies due to the certainty that the coloration indicate that the burning temperature was got above 500°C.

Postmortem Computed Tomography

Generally, the burnt bodies found are not destroyed completely but then also it is a very tough task to identify the victim, to know if the person was alive when fire started or was dead, the death cause, a third party involvement or intentional poisoning or intoxication. Firstly, the forensic team gathers the relevant and basic information such as the body position (found), its carbonization degree and its temperature. The needed toxicological tests for getting blood levels of CO and cyanide to confirm if the sufferer was dead when fire broke out are carried out by forensic pathologist. Further, various imaging techniques aid the forensic pathologist in identification and the autopsy guidance, fluid sampling and DNA sampling. The surgical dissection can get complicated by advanced carbonization state and foreign objects like bullets, etc. and bone changes like, fractures, etc. can be overlooked. Therefore, the modernized and improved cross – sectional imaging techniques, PMCT has replaced regular radiography and can give whole body volumetric study to aid the forensic squad in the investigation process. PMCT of a charred dead body gives certain imaging semiologist because multiple heat- related alterations are present. A sagacious approach is required for differentiating between general post – mortem changes and changes related to heat. Identification is among the first and important duties to be performed. If the victim has minor and superficial injuries of burn then the identification is easily done by the relatives but in cases of grievously burnt corpse, the identification is a very difficult task. External elements like jewelry, tattoo, watch, etc. are first collected and also the objects that are metallic (extremely radio opaque) and that is why are easily visualized on PMCT. Internal medical devices are of high use in order to relate them with alleged victim's medical records (vascular prosthesis, surgical clips, dental fillings, pacemaker, IUD etc.). In more severe cases, even the secondary sexual characteristics are in such damaged state that even the gender cannot be found. In skull, linear, fine fractures are the first heat – related lesions on the one that is exposed or revealed bone and are known as thermal cortical fractures. In completely burned limbs, thermal amputation (cut – off) along with transverse and smoothly margined fractures are shown by PMCT.

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The bone amputations look like a "flute mouthpiece" and are easily viewed on volume offering reconstructions. The heat causes internal bone structure modification (esp. for bone marrow). A pattern of spotted licenses in the bone marrow is shown by PMCT but only where there was direct exposure of fire on bone. MICRO – CT is among the advanced technologies to observe the thorough small material's morphology. It gives the surface layer 3 – D shapes of the fragmented bones and also let view its histological image, which is sliced with preparation of brittle, intensely burnt bones. The age is possibly estimated from the image that is based on alteration of its trabecular structures and then the overall morphology changes are compared by several life stages. Since the bone that is burnt is very frangible to mechanically cut, therefore this CT technique is very useful in observing the inside structure of the burnt bone. Also, the histological structures like haversian canals and osteons can be clearly visualized and in the bovine specimen, the lamellar pattern is clear. These are non – burnt bones' images but it is believed that a similar observation will be obtained in charred bones due to its high resolution (in single CT slices reconstructed 3 – D shapes) [1-9].

Methods of DNA Extraction from Charred Bones

In a study, several methods were performed on burnt bone samples for DNA extraction to ultimately aid in identification. The methods that were used are discussed below:

a) Firstly, the collected bones were properly scraped with a disposable scalpel for removing any sort of tissue remnants the outer surface. Additionally, use of sandpaper is made to clear out any dirt or debris from the outer surface.

b) After that, placed all bones in boiling water (time: approximately 20 minutes) and then used EDTA and a brush for their cleaning, before rinsing it by water and drying at 56°C for overnight.

c) These dried bones were then squashed in a fine powder to be further used in the extraction methods.

Organic Extraction Method

a) To 500mg of bone powder, the lysis solution was added and then was incubated overnight at 56°C.

b) {Lysis sol.: 600µL of stain extraction buffer + 40 µL of 1M DTT + 60 µL solution of proteinase K}

c) Once the incubation was over, addition of 60 µL of proteinase K was done to the sample after every 2 hours and further incubation was done (at 56°C) after each addition.

d) The process was repeated 3 times.

e) Then, spun the samples at 4000g in a micro – centrifuge for 10 minutes.

f) Next, 500 µL PCI (phenol/chloroform/isoamyalcohol) was put 3 times to the supernatant.

g) Spun the samples at 11000g for 5 minutes, after thorough mixing.

h) Took away the aqueous layer with care, placed on the Amicon filter columns and then rinsed thrice with the buffer.

i) Inverted the filter on microfuge tube (new) and then separated (via centrifugation) at 10000g for 5 minutes for extracting the DNA.

j) The DNA that was isolated was moved to new tubes (labelled) and stored for further analysis at 4°C.

Total Demineralization Extraction Method

a) Demineralization buffer (600 µL), 1M DTT (40 µL) and proteinase K (60 µL) were adding up to the burnt bone powder at 2 - hour intervals.

b) Mixed the samples by swirl at uniform intervals to make sure that there is no sample sitting at the very bottom.

c) Removed all kind of insoluble matter after centrifugation.

d) PCI was put into the sample and then separated by centrifuging at 4000g for 10 mins.

e) Transferred the aqueous layer in new tube.

f) Repeated this step thrice.

g) Lastly, concentrated the DNA using absolute ethanol (as in organic extraction).

Qiagen Kit Extraction Method

a) To 500mg of bone powder, added extraction buffer (300µL) ATL with proteinase K (20µL) and 1 M DDT (20µL) at 56°C for whole night.

b) Digestion was done by putting in Buffer AL and then incubating (70°C) for 10 mins.

c) Added 150µL 100% ethanol, and then centrifuged the formed mixture at 4000g for 2 minutes.

d) Transferred the supernatant to QI Aamp Min Elute columns, and placed in 2ml collection tube and further whirled at 8000rpm for 1min.

e) Discarded the flow through and transferred QI Aamp Min Elute column to new collection tube.

f) Before centrifuging at 6500g for 1 minute, washed it with 600µL of buffer AW1, 700µL of buffer AW2 and 700µL of absolute ethanol.

g) Dried the membrane of filter afterwards and eluted the DNA by addition of 30µL buffer ATE.

h) Incubated the column for 1 minute with lid closed, at room temperature and spun or whirled for 1 minute at 4000g.

i) Stored the extracted DNA at 4°C.

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These are some of the methods which were of high possibility of results in that study and once the DNA is successfully extracted, which is difficult in charred bones, the DNA analysis can further be performed as per the convenience. But it should be kept in mind that in case of charred bones, there are no sure chances of DNA extraction, it is only tried and if DNA is extracted then only further analysis is possible.

Conclusion

Finally, it is concluded that in case of severe charred bones, the identification is difficult as the DNA is degraded which further pose problem in the path of DNA extraction. Even if the DNA is extracted, there is no surety of getting the desirable results in analysis and identification. Charred bones go through various changes after burning like change in coloration, volume reduction, shrinkage, etc. which helps us to study the process it went through. PMCT may help to view various fractures and inner condition of the bone without damaging it. Further, various methods are there by which DNA extraction can be done if there are chances.

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