

CASE REPORT

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The effect of remaining in water in estimation of time since death in skeletal remains

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Abstract

Determining the time since death of an individual from skeletal remains is a challenging stage. In determination of time since death, climate conditions, environmental factors, conditions such as whether the body was buried or not, and genetic and personal factors must be evaluated all together. With these factors changing and with the exposure duration growing longer, changes occur in the morphology of the bone might increase. In this study, as part of an investigation, bones which had been found in a water-well during an excavation work in Mardin was used. After 36 pieces of bone that belong to a human were analyzed, it was found that the bones had lost most of their weight and density, that they were considerably fragile, that their medulla was emptied, and that there were intense erosions on the surfaces of bony tissue. While under normal circumstances and under the condition that it was a buried body it would take at least 50 years for these changes to occur, considering that the bones remained in water for a very long time, the corrosion had to be much more than expected. With the DNA comparison, it was found that the bones belonged to people who were claimed to have been killed in the 90's. In this study, it was aimed to determine the time of death of remains which were reported to have stayed in contact with water, and to study the effect of the environmental circumstances in which the bodies are found on the bones.

Keywords : Sirenomelia, prenatal diagnosis, first trimester**Introduction**

When a body is found, 3 fundamental questions are raised: who the person is, when s/he died, and how s/he died. Being one of these three fundamental questions, determination of time since death is a critical question, as well as being one hard to answer. When the level of decomposition of bodies is high or when full skeletonization occurs, variety of the rate of decomposition and postmortem changes complicate estimation of the time since death. Certain findings such as the activity and life cycle of insects in this stage lose their practicality as a long time passes after death [1].

There are 5 stages of decomposition of a body: Fresh, bloated, decay, post-decay, and skeletal [2]. While assessing the rate of decomposition in these stages, it is necessary to consider all together various criteria such as environmental conditions and the position of the body. A large number of factors such as the temperature, seasonal conditions [3,4], the weather being dry or humid [5], the rate of rainfall in the area, the area being in low or high altitude, the body being buried underground or on the surface, the body being inside a stream or still water, if left out, the body being directly under the sun or in woods, if buried, how deep it is, the pH ratio of the soil it has been buried in,

whether the soil is acidic or wet [6], the amount of ions in underground water, the vegetation, rodent or carnivore attacks, activities of the insects, the position of the body, whether there are any clothes on it or whether it is inside something, whether there is embalming, the size, weight and the health conditions of the body have impacts the development and level of decomposition [7-10]. Each feature, separately or associatively; cause various changes on the bone. As time passes after death, the data provided by these criteria for the estimation of time since death decreases. Besides, primary defects on the bone caused by factors such as blunt force trauma or gunshot injury also contribute to decomposition. In the presence of these kinds of trauma, it must be categorized as antemortem, perimortem, or postmortem with various criteria such as the characteristic and color of the trauma and the properties of the appearance of the surface of the wound [5,8,11,12].

The observing presence and development of insects and flies is one of the most useful methods, and there are a great many studies on this under various conditions [13,14]. When full skeletonization occurs, only a few insects can be seen. Even early period insects can be found in the cavities of bones. However, at a time when the soft tissue is lost and bones have a lack of water, it would not be beneficial as to the estimation of time since death [15]. Also plant roots and stems are seen superficially at the excavation area and this position they have is useful for estimating the time since death. An estimation based upon the positions of plants may hand in valuable results about the skeletonization

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process. For instance, examination of the plants growing inside the cavities of bones would be beneficial. Nevertheless, even this is an insufficient procedure in further stages [1,16].

Every estimation of time since death, in a context of forensic osteology, must start with macroscopic examination, despite all of its methodological limitations. However, in further stages, beside morphological methods, various chemical, physical and histological methods are also used when skeletonization occurs. A great many methods are used such as consistency and weight of the specimen, radiographic structure analysis, staining with Nile Blue and dichloroindophenol, reaction with mineral acid, reaction with benzidine, nitrogen loss, amino acid content of proteins, serological protein determination, degradation of lipids, remnants of fat-transgression, UV-fluorescence, radiocarbon method (^{14}C), Strontium-90 content, citrate content, analysis of luminol test, analysis of ^{210}Pb and ^{210}Po concentrations [17-27]. Nonetheless, using these methods is problematic, regarding both that they are highly costly, and that the preparation processes are too long. From the forensic osteological perspective, the very first stage must always be the morphological examination. Therefore, changes such as corrosion and erosion, which are caused on the bone by environmental and other conditions and which may advance depending of the time of exposure, must be assessed.

In this study, examining the bones that were found in water well in Kızıltepe, Mardin, it is aimed to estimate the time since death and to examine the effects of the environmental conditions on the bone with remains that have been reported to be in contact with water.

Case study

The case examined has been obtained with excavations conducted by the prosecution office in Kızıltepe district of the city of Mardin at the southeastern border of Turkey in June, 2013, as part of an investigation regarding the murders claimed to have occurred in 1990's. As a result of the investigation, the bones were thought to have belonged to people who had been claimed to be kidnapped, went missing, and thought to be dead. Thus, the bones obtained were sent to the Forensic Osteology and Odontology Division, the Council of Forensic Science so as to be examined in detail. The prosecution office requested the determination of how many people the bones belonged to, of their sexes, of the cause of their death, and if the bones had any findings that had evidential value regarding gunshot, torture, etc. Alongside the bones, also blood samples of relatives of the missing people were sent for DNA comparison.

In the records of prosecution, it was stated that the bones had been found in a water well (Figure 1). Following the examination of the bones, it was determined that they were highly worn-out, that there were erosion at their ends, and that their medullas had

enlarged due to postmortem impacts, and it was seen that there were postmortem fracture lines on the bones.



Figure 1.

It was determined that, of over 600 bones sent, most belonged to various animal species. The morphological features, cortex thickness, and the medullary canal width of the bones available were evaluated, and on these criteria were they discriminated on the categories of human and animal. After separating these bones, with the evaluation of human bones, it was reported that, according to the morphological structures of the bones, they belonged to 5 individuals, one being a female, two being males, and two being children.

Following the examination of the bones, a left-side tibia, 2 pieces of bone that belonged to fibula, 2 pieces of costa, the 2nd cervical vertebra with 1 piece of vertebra, 1 femur head, 1 piece of acetabulum, 3 tabular pieces of bone the belonged to the skull, 13 bones of metatarsals and metacarpals were determined to have belonged to an adult. Of the bones that were categorized as children bones, it was seen that there were 2 pieces of tibia, and pieces of epiphysis that were separated from an epiphysis line belonging to two different femur heads. Little bone fragments were also seen. It was assessed that the morphological structures of the bones were highly fragile, and that the cortical bone structures were advanced corrosion (Figure 2).



Figure 2.

It was stated in the report that, of the two sets of bones belonging to children, one may have belonged to a child between the ages of 4-8, and the other, 8-14. However, since the individuals reported as missing were adults, a piece of tibia and two metacarpal of the adults were chosen, as it was observed that these bones preserved better the bone morphology for DNA analysis.

DNA isolation was done by using the method of phenol chloroform on the biological samples with the DNA Isolation Experimental Method. The amount of DNA was determined with Quantifiler Duo Kit instructions. The isolated DNA was reproduced with the instructions of MT Investigator ESSplexPlus Kit PCR by the method of PCR. The polymorphic STR DNA regions were typed with the capillary devices of ABI 3130xl and the software GeneMapper ID by the Method of Capillary Electrophoresis and the Experimental Method of Determination of Length Polymorphism. Autosomal examination of STR DNA was done with Essplex, Idplex, and Minifiler kit, and the examination of Y-STR DNA was done with Y-Filer kit.

As a result of the DNA analysis, a DNA profile for a female from the tibia, and two different DNA profiles for two males from the metacarpal were reached. Following the matching of bones with the blood samples, it was determined that, regarding the Y-STR, metacarpal sample no. 1 may have been an individual in the same pedigree as people whose blood samples were taken, and that metacarpal sample no. 2 may have been father with 99,99% probability to another person whose blood sample was taken.

In the case, wherein it was requested to determine the time since and the reason of death alongside the biological profile, the greatest limitations of the work were that the bones were not complete, and that there were intense losses of tissue. Moreover, that no sample of dirt was sent during the scene investigation prevented a dirt analysis and toxicological investigation. Along with the morphological changes that occur on bones in time, also the loss of weight and intensity, and the degree of the post-mortem corrosion on the bones are used for determining the time since death. However, as the environmental and climatic features of the region, as well as the morphological features of the dirt in which the remains were found, affected the degree of the changes in question, the bones available were disintegrated and suffered intense tissue loss, preventing the determination of post-mortem interval.

Discussion

Estimation of time since death is one of the most important issues for forensic sciences. With the cases, over which 24 or 48 hours pass, the estimation of time since death hands in better results. As time passes after death, alongside the analysis of physical changes on the body, other techniques also start to be used. Criteria such as disruption of clothes and activity of insects

provide help as to the estimation. As skeletonization begins, the effects of these factors start to diminish. In this stage, histological, chemical or physical methods can be helpful [16].

Regardless of which method is used, it is necessary to evaluate the effects of environmental conditions on the bone. Bones that belong to the same person might display different features with different burial and different natural conditions. The body being in woods, indoors, on an open field, or in water causes different results [15]. For instance, a body left on an open field in hot and humid weather decay fast, while the course of decomposition of a body left on an open field, but this time in cold weather, may take longer. When the case is skeletonized remains, it is necessary for better results to know which conditions the bones were exposed to, and how long.

In consequence of that there is water in the environment of the body, or that the body is left in water, intense erosion and corrosion will occur on the bones as time goes on, and as water draws back. The most significant change on bodies that remain in water is formation of adipocere. Adipocere is a type of decomposition resulting from saturated fatty acids as a result of hydrolysis and hydrogenation of neutral fats in the body. Although it is often seen in bodies that remained in water for a long time, it can be observed various environmental conditions. For one thing, in burials, adipocere may form under many conditions that are affected by factors such as the pH value of the soil, heat, humidity, and the amount of oxygen in the graveyard [28]. Factors such as body fat index, humidity, medium alkaline level, the temperature of heat, anaerobic conditions, and presence of the appropriate bacteria create a convenient environment for adipocere formation. Whether the water is a stream or a still water, and the pH value of the water affect the decomposition rate of the body [3,29,30].

Since external microorganisms cannot pass through air-proof spaces, further bacterial decomposition stops at the adipocere stage. The fats, which come to have the stiffness of paste, solidify, preserving the body for years [31]. However, when the body is exposed or the factors causing adipocere disappear, there starts a rapid decomposition. In the next stage, the bright look of the bones often disappears [5].

In the case we investigated, the bodies were reported to have been found in a water well. The well, which had probably had water in it when the bodies were dumped, dried up over time, and the bodies started to contact with the air. Regarding that the region wherein the well is located is hot and dry in summer and cold and wet in winter, probably a rapid process of erosion occurred. Regarding the weather conditions, within the 20 years following death the well was filled and emptied again and again, the bodies –and the bones after them– were exposed to this cycle, and it is thought that the rate of erosion increased accordingly. Taking the human bones into consideration together with the animal bones, it is

conceived that the bones were exposed to animal attack. Gathering all of these factors together, since the time the individuals had gone missing who were identified by DNA comparison is known, intense erosions and tissue losses occurred on the bony tissue since the time they went missing –which is 20 years ago– until when they were found. However, due to the advanced level of erosion detected on the bones, following a macroscopic examination, it was assessed that the appearance of the bones causes an image of bones with a longer time passed after death.

Conclusion

Regarding that the stages of decomposition are shaped by environmental conditions and the properties of the body, each body must be evaluated according to the environmental conditions where in it is found, and the destruction on the bones caused by all of the factors must be taken into account while estimating the time since death. Just as in this case, in some cases the bones may erode much more than they would under normal conditions. In such cases, it would be misleading to appeal to an assessment reached by the morphological properties of the bones. Conducting further investigation by applying to physical and chemical methods should prevent reaching incorrect conclusions. Also in such cases as this one, which has a claim of murder, even if the bones display a very old or archeological appearance, a molecular genetics investigation must be conducted for identification, excluding the possibility that the bones may belong to that person.

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