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Identification process of skeletal remains from mass graves: Our experience and proposal guidelines

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ABSTRACT

Aim of this paper is to present our experience and proposal guidelines for reducing the number of samples for DNA analysis of skeletal remains from mass graves, whether for scientific purpose or for the identification of mass graves victims. Therefore, the analysis of 94 bone fragments included the following measurements: femur length and the femoral head diameter, the diameter of the upper, wider portion and lower wider portion of the bone fragment, densitometry of the fragments and measurement of mass and volume of fragments. Bone density was determined on the basis of measured values of mass and volume. The results of fragment matching by physical analyses were compared with the pairing results obtained by previously conducted DNA analysis. Deviation in measured values of matching bone fragments that made a pair was calculated for all successfully matched fragments. By the results of DNA analysis 36 femoral pairs were successfully formed. Measured values were added to the DNA analysis. Out of 36 pairs, positively ascertained by the DNA analysis, 29 pairs were formed after adding the results of physical measurements and removing the data where femur samples were damaged. Total correspondence in measurements of the femoral length was noted in 25.9% pairs, while the correspondence within the 5% error was 100%. Density of the tested femurs was significantly different for the same person (DNA match), both for the left and the right femoral fragment. It would be optimal to choose only the whole-length left or right femur and thus reduce the number of samples by 50%. With regard to the results of our research and the observations deriving from them, as well as to the guidelines we used in the study, we suggested these guidelines be used both for scientific researches and to identify mass graves victims.

1. Introduction

Man has always asked questions about the essence of his existence and the secret of life he has been holding. The need to understand urges him to do continuous researches, incites philosophical and religious debates and scientific objectification of the macro and micro world. Identifications of human remains are often time consuming and complex processes. Methods of identification should be planned, diverse and complementary to increase the number of efficient identifications and fulfill the main objective - to show respect for the deceased and return them to their families [1,2]. The protection of human rights of both living and deceased persons, will be supported and passed on to next generations in this way.

One of the major achievements of molecular biology in forensic medicine is the identification by DNA typing of biological samples. This particularly relates to the identification based on bone or teeth samples taken from the people killed in mass disasters or from the exhumed bodies, which cannot be performed by any other standard method [3–8]. The DNA analysis is a very useful and certainly the most precise current method of identification in such cases. The analysis is based on the comparison of the DNA. Human DNA, isolated from skeletal samples (bones or teeth), is compared to the DNA isolated from blood samples, buccal mucosa swabs, hair and the like, taken from the presumed closest relatives [3,9].

There is a number of published researches dealing with the problems of identification of victims found in mass graves after the Homeland War, World War II and even after World War I [3–8,10]. Due to the difficulties of isolating DNA from such samples,

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the majority of authors treated the problem of the DNA isolation while only a small number engaged in the proper selection of number and type of bone samples for the DNA analysis.

The success of any forensic identification depends to a large extent on the range and preservation of the data collected in the field [3,11]. Warring parties use different methods to hide war crimes and the location of the burial: excavation and relocation of remains from one place to another; further relocation to tertiary sites; disassembling and mixing of parts of the body, compacting and crushing. All such activities complicate or make it impossible to determine the number of bodies, their assembling and identification [3,8,12]. Consequently, the number of fragmented bone samples is dizzily rising. The cost price of the DNA analysis would be extremely high in such situations and the DNA identification could not be made for all samples.

The ideal situation with preserved skulls, teeth and the corresponding number of femur pairs is rarely found. If the identification is performed only on the basis of the femur it seems logical to halve the number of samples and restrict the DNA analysis on left or right femurs only. Although it is relatively easy to morphologically distinguish larger fragments of the left from the right femur, the problem arises when some of the femurs are missing (either the left or the right one) or if they are fragmented to such an extent that they are not comparable.

Our idea was to explore the possibility of distinguishing and matching femurs of the same person on the basis of their size and physical properties after they were previously DNA analyzed and paired (left/right) with certainty. Therefore, we checked the usability and features offered by available and not overly expensive physical analyses used in the process of pairing left and right femur fragments from which samples would be taken for the confirmatory femur pairing DNA analysis.

The second objective of this study was to present guidelines/standardization of skeletal remains from mass graves based on our experience.

2. Experimental

All studies were approved by the Ethical Committee of the University of Split School of Medicine (No. 32-1/06). In the study we analyzed the femurs found in bone fragments, exhumed in mass graves on the island of Daksa, near Dubrovnik, in September 2009. They were previously linked by the DNA analysis and some of them were identified [13]. Correspondence between bone fragments (for all successfully matched bone fragments) has been expressed by statistical calculation as the measure of success of physical analyses in pair-forming. The result is the optimization suggestion for the DNA analysis of the bone samples number.

The treatments of the mass grave and positive identifications have been presented in our previously published study [13]. The location was processed complying with standard archeological proceedings. Basic anthropological tests were performed to determine the minimum number of victims, their gender, height and age at the moment of death. Bones with pathological and traumatic changes were identified. The DNA was extracted from bone samples and from blood samples of the presumed relatives. To determine relationship between the victims and their potential relatives we used the AmpFlSTR Yfiler PCR Amplification Kit, and MiniFiler PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA).

2.1. Material

About 10,000 bone fragments were found and singled out at two locations: 53 skulls (32 in location I and 21 in location II); 104 femurs (64 in location I and 40 in location II). The treatment of 104 femur specimens was closely examined in the study. Considering the number of the skulls found, we assumed that there were 53 victims buried in the grave (all of them men). Femur samples were chosen for the analysis because of their solid structure. Because they are often well-preserved, the high quality DNA can be successfully isolated most of the time. The number of samples for the DNA analyses can potentially be halved by matching the left and the right femur and further analyzing only one femur per victim. The procedure of the whole treatment and physical analyses of femur samples is shown in Fig. 1.

2.2. Methods

After examining the bone fragments we created a database and measured the following values: femur length (L) and the femoral head diameter ($Ø$). In our previous study, we used Trotter equation for calculating the body height [13]. Very often we only have parts of the femur. In such situations, there is no reliable way to pair with certainty a left and a right femur by measuring. However, we tried to reduce the number of samples for further, more expensive analyses, by applying simple physical measurements. So we decided to make measurements on fragments excluded from equal positions, to simulate the situation in which we do not have the whole femur. Parts of the femur were then isolated at the 10% length of the total femur length and measured at the distance of 20% from the cranial end (Fig. 2). Measurements were performed on a personally constructed anthropometric table. Further analyses were made on these isolated fragments of the femur: measurement of the outer diameter of the uttermost bone fragments, physical values: weight, volume and surface density of bone fragment.

2.3. Outside diameter of the uttermost parts of femur fragments-measuring by caliper

Measuring was made by the caliper with an accuracy of ±1 mm and the measuring range of 0–40 mm. The following variables presented in millimeters (mm) were measured in all femur fragments: the diameter of the upper, wider portion of the bone fragment ($D1$); the diameter of the upper, narrower portion of the bone fragment ($D2$); the diameter of the lower, wider portion of the bone fragment ($D3$); the diameter of the lower, narrower portion of the bone fragment ($D4$) (Fig. 2).

2.4. Weight measuring of femoral fragments

The weight of all femur fragments is shown in grams (g) and measured by the analytical balance with accuracy ±0.1 mg. The values measured were then entered in a previously constructed database.

2.5. Density measuring of femoral fragments by densitometry

The bone mineral density (BMD) of all femoral fragments was measured by a quantitative method – densitometry. The BMD was expressed in grams per centimeter squared. The densitometer model QDR 4500 C (S/N 48 034; Bedford, MA 01730, USA), was used for this purpose and the measured values entered into the database.

2.6. Volume measuring of femoral fragments

The volume of all femoral fragments was measured by the laboratory cylinder with the measuring range up to 250 ml and expressed in cubic centimeters (cm^3). Fragments have been immersed in cylinder filled with distilled water and left for 24 h.