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## **Forensic analysis of incinerated human remains using anthropological and genetic approaches**

### **Forensic case presentation**

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## SUMMARY

*We present the "Mariachi case" which is a representative case with a history of incineration, referring to a victim of Femicide, who entered Forensic Research Instituto (IDIF) at La Paz, Bolivia, presenting remains composed of ashes and few fragments of barely analysable bones, whose identification was not possible using method of Legal Medicine; a study model was applied, proposed by the Forensic Anthropology and Genetics Laboratory, through trials in 54 cases analyzed during 2007 to 2017.*

*We propose suggestions for the systematization of the study and the approach in cases of incinerated human remains, which begins with the joint assessment of the samples under the criteria of Anthropology and Genetics, where the result of the process lies in the search and selection of the sample analyzable that provides the possibility of establishing the genetic profile; likewise, the selected sample(s) must provide information related to the identification quatrains, whose goal is to know their identity and other aspects related to their death.*

**Keywords:** *Incinerated human remains, protocol, criteria, characteristics, genome, autosomal DNA, genetic profile*

## CASE PRESENTATION

The "Mariachi" case was very representative, given its connotation and complexity, related to a femicide investigation; it entered into the IDIF in 2015, belonging to a young mariachi singer, whose remains were collected by the Police with signs of incineration consisting of ashes and few fragments of bones, which were packed in a Kraft paper envelope (manila), where the main question of the family and the authorities, was to know who it was.

The criteria for the selection of samples, after joint observation of the evidence of the Mariachi case were: size, magnitude of the burn, tissue alteration and topographic location of the bone with respect to incineration.

The samples selected were fragments of skull bones (vertical portion of the frontal and other flat bones), the largest of 4.5 x 3.8 cm and smaller ones, such as bones of the pelvis and diaphysis of fragmented long bones, that preserved human characteristics and allowed to establish sex and age with certainty, through the anthropomorphic protocol (figures 1 and 2).



Figure 1. Image of incinerated bone fragments extracted from the ashes; 6 of them retain analyzable characteristics



Figure 2. Image of a fragment of pelvis with signs of extreme incineration; the greater sciatic notch shows feminine characteristics (one of 6 selected bones)

The fragment of cranial bone presented a region that conserved tissue that could be analyzed by Genetics; after being photographed, measured and inventoried by anthropology, it was

immediately transferred to the genetics laboratory for processing in order to have results simultaneously to compare findings (figure 3).



Figure 3. On the left side we have an image of one end of the long bone fragment with signs of incineration. On the right side, we have bone fragments (long bone and flat bone) valued by genetics with parts of bone that can be analyzed.

In this particular case, the fragments of bone found presented serious deterioration; only 1 of the samples conserved enough DNA to establish identity.

After the application of the joint procedure of Forensic Anthropology and Genetics of IDIF - La Paz in the present case, it was determined that the samples examined by anthropology did correspond to a woman young of Latin traits; also, the results of the Laboratory of Genetics in the sample analyzed (fragment of bone) concluded it was related to the parents of the victim, obtaining a result of 99.99% precision. The protocol concluded with reporting by area and a joint report by both Anthropology and Genetics services establishing the identity of the mariachi case.

## DISCUSSION

The forensic study of incinerated human remains constitutes a challenge in the investigation of identity; IDIF Bolivia La Paz (pilot center) has been facing this

type of exams since 2007 thanks to the implementation of the Genetics and Anthropology laboratories, having a casuistry of 54 cases until 2017; in all cases there was a deep deterioration of the identifying characteristics in face, hands and body, leaving fragmented human remains; our procedure is established for revealing the identity, by means of the anthropological identification and the genetic profile, so they can be used in Court.

The analysis of cases with complex conditions such as incineration, mutilation, skeletonization or others was usually made by the Anthropology and Genetics laboratories individually, until the Mariachi case (2015), which demanded joint intervention from both laboratories; all the available methods were applied in order to determine mainly their identity, concluding in the need to have a joint study model for this case and in the future for others.

The procedure began with the receipt of information by the requesting authority and the police (investigator of the case), with information on the removal

of the human remains and the points of expertise, in order to contextualize the case. It is important to obtain the antemortem data of the person sought or disappeared; the participation of the relatives is suggested, who in turn provide reference samples (blood, gingival swab or others).

Other data of interest are the date and circumstances of disappearance (if any), the description of the clothing at the time of disappearance (due to the finding of textile residues or fibers in the scene or the human remains), prosthesis, surgical antecedents, fractures, dental and photographic records, etc.

Handling the samples, the chain of custody must be cautious and mandatory. The laboratory should be prepared, anticipating the genetic contamination, before the entry of the samples.

The fundamental part of this procedure consists in the joint examination by both experts of Anthropology and Genetics at the reception of the samples; they are dealing with ashes and few brittle fragments of bone; in this case, there were at least 6 samples that preserved characteristics that could be examined by anthropology, of which only 1 (one) was suitable for genetic analysis.

The laboratorial procedure by anthropology includes:

- The count of bone pieces, reconstruction of segments, photographed and recorded, radiographic study of segments (skull, thorax, pelvis), cleaning of remains avoiding the use of chemical and physical means as well as microscopic study of the fragments (stereomicroscope).

- Determination of: Species, identification quatrains, trauma or pathology.

According to the morphological examination of the bone remains, it was possible to determine the bioanthropological traits, considering the following:

- Determination of sex. For this estimation the bones to be examined are the pelvis, the skull, the femur or the humerus; it is suggested to include the head diameter.
- Determination of ancestry. The morphological characteristics of the skull, the configuration of the nose, nostrils, nasal spine, orbits, sutures, the presence of the teeth in the blade or Carabelli's tubercle should be included; it is recommended to search for skin, hair and clothing remains; the metric and cephalic indices are not possible in these cases due to the disintegration of segments.
- Age. It can be established by the development and ossification of the bones in the skull, observing the degree of obliteration or closure of sutures, the dental study (wear and translucency) by the methods of Gustafson and Lamendin for adults and Dermijian in subadults; the study of the pubic symphysis as well as the study of the surface of the atrial facet of the fourth rib.

- Size estimation is not possible because they are human remains (fragments).

The traumatological examination does not provide elements, due to the characteristics of the samples examined (fragments of bones) subjected to the action of fire.

The samples selected by Genetics, were transferred with chain of custody to the laboratory to determine the genetic profile (DNA), performing:

- The treatment of bone samples (cleaning, washing, spraying).
- DNA extraction.
- Quantification of DNA
- DNA amplification: using gene amplification techniques, which allows obtaining specific regions of DNA.
- Analyzed polymorphisms: by the analysis of fragments of type STRs for 16 loci of the genome, the analysis of autosomal nuclear DNA.

The methods used by Genetics, were the least aggressive, taking into account that the sample analyzed was minimal (scarce). It is advisable to perform DNA isolation procedures from bone remains with samples obtained in compact bone tissue such as diaphysis of long bones and in dental pieces where DNA is better preserved; soft tissue analysis is not recommended due to the history of alteration due to the action of the harmful element (fire). The determination of sex is made in all the samples that are analyzed by amplifying a short sequence of the gene of amelogenin which in the X chromosome has a length of 106 base pairs and in the Y chromosome 112 base pairs.

The human remains considered complex (incinerated, fragmented or others), should be analyzed and solved by a scientific expert team with suitable professionals and sufficient experience for it; there must be laboratories and the necessary equipment, where the main objective is to achieve identification; for this purpose the implementation of a joint action model is proposed, it provides more reliable results which may be presented as evidence to Court.

The proposed procedure allows to include or exclude samples (human remains) within an investigated case, by determining species, sex, age and / or racial affinity; occasionally, remains may be neither human nor from the sought gender; another important aspect is the establishment of the number of individuals.

Genetic studies should be cautious; they have fragmented, highly altered samples; the use of the analysis of fragments of type STRs for 16 loci of the genome and the analysis of the autosomal nuclear DNA is highly recommended.

Given the usual deterioration, soft tissue is not suggested bone tissue is preferred since it preserves DNA in a better way.

It is important to have the support of family members and authorities (prosecutor, police, witnesses, etc.) throughout the procedure; the best results are achieved when they provide information, reference samples or others. The final recommendation, after our experience (Anthropology and genetics) is to form scientific teams that contribute to justice in an integral and joint way, providing all the necessary criteria.

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