Human identification with combined anthropologic and genetic tools: two case reports in forensic medicine practice

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Abstract
The identification of skeletonized corpses or skeletal remains, in forensic medicine practice, as well as being an imperative to human rights respect, has particular relevance and significance in psycho-social terms. If, on one hand, the Universal Declaration of Human Rights advises identification before all body inhumations, on the other hand, returning the identity to an unidentified corpse and subsequent return of the corpse to his family is, itself, the instrument which will allow for the possibility of beginning the family grieving.
Forensic anthropology is the first approach towards the identification of unknown skeletonized corpses, which may lead to positive identification of the body or, sometimes, cannot go further than establish some pointers that will lead to further attempt to genetic identification.
Here we report two cases of two dead bodies, unidentified, that were presented to the National Institute of Legal Medicine (INML), with instructions, from the prosecutors, to be determined possible cause and circumstances of death and achieve to individual identification.
Forensic anthropology, allowed, in both cases, some indications that pointed to alleged missing individuals.
Genetic study of skeletonized bodies and family members of the alleged missing individuals has turned possible, in one case, the positive identification. For one of the corpses, most likely due to the weather conditions to which he was exposed for a very considerable period, it was not possible to achieve to positive identification. However, in the latter case, we believe that the use of mitochondrial DNA study may help to settle the case with positive identification of the corps.

Keywords: Human Identification, Forensic Anthropology, Forensic Genetics

Introduction

From forensic anthropology point of view, the study of a skeletonized body or skeletal remains, try to determine a set of characters which individualize that skeleton, in order to establish an identity (Cunha, 1996). However, establishing the identity of skeleton or skeletal remains, using exclusively anthropometric parameters is not possible in all cases. Forensic genetics analysis complements the anthropological examination and leads to the establishment of individual identification, without which, according to the Universal Declaration of Human Rights, no human body should be buried.
In the nineties of the twentieth century, microsatellites or short tandem repeats - STRs began to be used in genetic identification due to its high level of polymorphism (Weber & May, 1989, Edwards et al. 1991). The genetic profile of an individual is defined by the number of repetitions that each STR presents for a set, as wide as possible, of STRs.
The study presented here reports the attempt to identify two unknown corpses, which were taken to INML with a request, from prosecutors, to determine the possible cause and circumstances of death and identification of the corpses. One of the corpses was found accidentally and the other was collected under a criminal investigation by the Portuguese Criminal Police (PJ). Individual identification, based on anthropological and genetic studies, was possible in one corps.

Material and Methods

Unknown corpses

Two unknown dead bodies, one occasionally found in a zone of salt water and the other one found in the wake of a judiciary inquiry, in a wooded area.
Corps of the salt water zone - dead body found in a lateral position, with much of the body submerged and only visible its right side (arm, torso and leg). Death was verified by a doctor of the Health Authority.

Corps of the forest zone - skeletonized body (Figure 1), retaining some mummified areas, collected by the PJ, under a forensic investigation, in a wooded area.

Fig. 1 - Skeletonized body. Corpse found in the forest zone.

Anthropological Exam

The studies were held with the purposes of (1) description of the skeleton and taphonomic alterations; (2) definition of a biological profile based on parameters (2a) sex, (2b) age at the time of death, (2c) height and strength, and (2d) population affinities; (3) factors of individualization; (4) causes and circumstances of death.

Description of the skeleton and taphonomic alterations

The examination at the macroscopic level was chosen to evaluate the taphonomic processes involved in studied skeletal remains. Assessing and recording the taphonomic alterations, was achieved by taken into account variations in color, texture and shape, as well as *peri-mortem* and *post-mortem* fractures, against *ante-mortem* pathological trauma.

Sexual diagnose

Performed taking into account the sub-pubic angle, shape of ischiopubic ramus, occipital robustness and glabella prominence (Buikstra & Ubelaker, 1994).

Age at time of death

Determined by analysis of the state of the teeth and eventual alveolar reabsorption, evaluation of the metamorphosis of the pubic symphysis (Mendonça, 2000) and obliteration of endo and exocranial sutures (Buikstra & Ubelaker, 1994).

Stature and robustness

By the maximum length of the femur, we determined height (Mendonça, 2000). The robustness was determined by general overall complexion of the skeleton.

Population affinities

Determined by evaluation of facial features, mainly nasal features, inter-orbital space and presence/absence of prognathism.

Cause and circumstances of death

The study was conducted to screen degenerative, congenital or neoplasic pathologies and bone fractures.
The screening of degenerative joint disease, was focused on different bone elements involved in the major joints of the appendicular skeleton, as the shoulder joints, elbow, wrist, hand, hip, knee, ankle and foot. The study also through observation of the articular processes of the vertebrae and the examination of the body of the vertebrae.
To screen for congenital pathology, attention was given to craneosinostosis, specifically the scaphocephaly, and screening for spina bifida occulta.
In order to screen neoplasic diseases we only search the presence of osteomas and characterize the type of bone reaction.
The analysis of the bone fracture was made at the macroscopic level. In order to provide a glimpse of the bone fractures, we recorded all information concerning the skeletal location of the fracture and their characteristics, such as type, alignment features, state of healing and possible signs of infection and/or other alterations.

The paternity probability of the alleged missing person relatively to the indicated son was 99.99999950%, which corresponds to practically proven paternity.

Corpse of the forest zone

Skeletonized body, retaining some mummified areas, including the genitals and the proximal portions of the femur. The hip joints were maintained in situ and still inside the clothes.
Complete skull, skeletonized, retaining a minimal portion of the frontal scalp.
The complete skeletonization of large parts of the body and the mummification of the mentioned bone pieces, suggest a time elapsed since death of more than 12 months.
Narrow and small pelvis, thick ischiopubic ramus, narrow sub-pubic angle, narrow greater sciatic notch. Prominent glabella, notorious occipital muscle insertions, notorious occipital crest, developed mastoid apophysis. All this set of characteristics point to a male person.
Sternal end of fourth rib in phase IV, by the scale of Iscan and Loth (Hartnett, 2010). Clavicles with ossified sternal ends, which indicates an individual older than 30 years. Auricular surfaces without degenerative changes. Pubic symphysis in Phase III, by the scale of Suchey-Brooks (Hartnett, 2010B). Vertebral bodies without degenerative changes. Single-rooted teeth without root transparency. Cranial sutures obliterated.
This set of features indicated a relatively young adult male, with more than 30 years and less than 40-45.
Small femora. Physiological length of 426 mm and maximum length of 433 mm. Individual with low complexion, not robust and stature between 160.6 ± 90 cm.
Narrow face, narrow and deep palate, undershot jaw, long and narrow skull. Narrow nose and face, pointing to Caucasian individual.
Slight deviation of the bones of the nose, including the left nasal bone, forming a callus.
Sternal drilling (morphological).
Traumatic injuries were not detected. None of the ribs, vertebrae or other bone displays *peri-mortem* lesions. Hyoid bone was not recovered, only a fragment of ossified thyroid cartilage which does not allow any kind of credible inference.
The biological profile inferred based on the anthropological examination was consistent with an alleged victim indicated by the PJ.
From the blood stains from brothers and mother of the alleged victim, genetic individual profiles were obtained. From several teeth and several fragments of the femur of the corps, and after several attempts, it was not possible to obtain a complete individual genetic profile (Table 2). We cannot calculate biological kinship parameters or positively identify this body with autossomal STRs studies.

Tab. 2 - Genetic profiles of the alleged victim and his mother and his brothers in different STRs studied.

	Perfis Genéticos			
Genetic Profiles	**Mother**	**Brother**	**Syster**	**Alleged victim**
D3S1358	15/17	15/17	17/19	15/-
TH01	9/9.3	6/9.3	6/9	7/-
D21S11	29/31	29	29	-
D18S51	10/17	15/17	15/17	-
PENTA E	12/13	13/17	7/13	-
D5S818	10/11	11	10/12	12/-
D13S317	11/12	8/12	8/11	-
D7S820	10	10/11	10	-
D16S539	11/12	11/12	11/12	-
CSF1PO	11/12	11/12	11/12	-
PENTA D	10/12	10/12	10/12	-
Vwa	14/17	14/16	16/17	-
D8S1179	10/15	10/15	10	10/-
TPOX	8	8	8/11	-
FIBRA	19/22	19/20	20/22	20/-
D2S1338	17	17	17	-
D19S433	12/15	15/15.2	12/15.2	-
AMELOGENIN	X	XY	X	-

Conclusion

With anthropological examinations and genetic markers study it was possible to positively identify the unknown corps found in the salt water zone. It was not possible to get a complete genetic profile for the body removed from the wooded area. The complete skeletonization and exposure of bone remains to several factors and weather conditions during a long period of time, probably more than one year, made irreparably DNA damages.
In most human cells, the mitochondria contains 103-104 copies of it's circular genome (Iborra et al., 2004). The circular form of mitochondrial DNA gives it also the advantage of resistance to degradation, so, it becomes the choice for the analysis of this kind of ancient/degraded DNA. In an attempt to resolve the case of the corpse found in a wooded area, with genetic studies, would be useful in future studies the analysis of mitochondrial DNA (Tex et al., 2010).

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VOLUME I NR. I MAY 2011

ISSN 1338-4589

Folia Societatis Medicinae Legalis Slovacae

Vedecký recenzovaný časopis Slovenskej súdno-lekárskej spoločnosti Slovenskej lekárskej spoločnosti

Refereed scientific journal of The Slovak Society of Forensic Medicine of Slovak Medical Society