Case Report

Positive Post-mortem Identification of Skeletal Remains of a Person Using Radiography and DNA Analysis: A Case Report

Hossein Bahadoran[1], Sajad Moghadami[1], Ali Miri[2], Sajjad Habibi Azarian[2], Reza Asghari[1*]

1. Department of Anatomy, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Human Genetic Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

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Citation Bahadoran H, Moghadami S, Miri A, Habibi Azarian S, Asghari R. Positive Post-mortem Identification of Skeletal Remains of a Person Using Radiography and DNA Analysis: A Case Report. Anatomical Sciences. 2023; 20(1):31-38.

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Article info:
Received: 27 Apr 2022
Accepted: 20 Jun 2022
Available Online: 01 Jan 2023

Keywords:
DNA analysis, Short tandem repeats (STRs), Anthropology, Post mortem (PM), Ante mortem

ABSTRACT

Introduction: This study surveys the results obtained from a missing person (for 40 years) from the Iran-Iraq War in Majnoon Island.

Case Report: Skeletal remains were examined by anthropometry. Parts of selected femur and tooth samples were sampled for genetic identification. DNA extraction from bone samples was performed using the QIAamp DNA Blood Mini Kit with a slight change. DNA was extracted using the Qiagen column, washing buffer, and bind-elute technology. Extracted DNA was quantitated by Quantifiler™ Trio DNA Quantification. According to DNA concentration, AmpFℓSTR Miniifiler PCR Amplification or AmpFℓSTR Identifiler PCR Amplification kits were used for genotyping. FTA cards were used to store and extract DNA samples from the person's family. Genetic profiles were prepared using the AmpFℓSTR Identifiler Direct PCR Amplification Kit. The genetic matching of sex chromosomes was investigated with Yfiler™ PCR Amplification and Investigator Argus X-12 QS kits. Genetic matching and kinship calculations were done using Familias and NoorGIS applications.

Results: The radiographic and genetic examinations ultimately confirmed that the dead body matched the relevant family. Despite the challenges with DNA-degraded samples, a good genetic profile was obtained for genetic identification.

Conclusion: Anthropometric examination, especially post-mortem evidence (e.g. radiography) and genetic examination confirmed that the dead body matched the relevant family.

*** Corresponding Author:**
Reza Asghari, PhD.
Address: *Department of Anatomy, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
E-mail: Rasghari57@yahoo.com

Introduction

The Iran-Iraq War (1980-1988) resulted in military and civilian casualties [1, 2]. Among the dead, like many other wars, a significant number of bodies were missing through not being recovered and identified. At the disaster victim identification (DVI), Matching to post mortem (PM) evidence with the antemortem (AM) data in various methods, such as evidence-based methods, medical history, fingerprinting, etc. may help to identify missing persons [3, 4]. Process is the collecting of the antemortem data of the announced missing persons [5]. For instance, the importance of storing and interpreting radiographic medical data correctly highlighted in medical histories [6]. Frontal sinus pattern matching is a practical means of forensic identification [7]. Dental structures are the hardest and well-protected structures in the body. These structures resist decomposition and high temperatures and are among the last ones to disintegrate after death [8].

Also, DNA analysis is a method that can scientifically help identify a large number of missing persons in an event, such as war [9, 10]. The development of DNA analysis techniques served to undermine forensic anthropology's classic role as a field that exclusively focused on victim identification [11]. This method's strength and value have grown steadily due to the development of short tandem repeat (STR) and single nucleotide polymorphisms (SNPs) multiplexes. Besides, using STR markers for investigating X and Y chromosomes has also added more credibility to genetic identification [12-16].

Efforts continue to identify and locate the missing citizens related to the conflict. Personal history, fingerprinting, and public documents were among the numerous evidence-based tools to discover missing people. The Search and Recovery Committee (SRC) identified missing persons during the war. Excavations of missing persons from the Iran-Iraq conflict have led to the finding of 3448 unidentified persons.

Case Report

This case was one of the Iranian soldiers missing in the Iran-Iraq war from Majnoon Island. The missing person was Identified according to anthropology and DNA analysis and was confirmed with the post-mortem method. Skeletal remains were found and collected and identified 40 years after the war today using genetic identification [17]. This case's identification was confirmed using the supplementary Yfiler and Investigator Argus X-12 QS Kits and Minifiler /Identifiler kits [18-20].

Handling of skeletal remains

The SRC excavated scattered islands in the border regions of Iraq (Figure 1), which include plain, alluvial, and salty areas. The age and height of the skeletal remains were evaluated through anthropology. Then, three teeth and four CMs of the femur samples were taken and sent to the DNA analysis laboratory. Samples are stored at the laboratory of Noor Center in Tehran, Iran, for further investigation.

Analysis of anthropology

Morphological and anatomical examinations showed that these skeletal remains weren't commingled. The pelvis bone and sacrum were used to determine sex. The greater sciatic notch, the acetabulum, the ischial spine of the pelvis bone, and the sacrum's landmark (promontory and the comparison of length and width) have been examined.

The humerus's accurate examination, the iliac crest, the ischial tuberosity, and thoracic and sacral vertebrae indicated that he was 18-20 years old. Using the femur, tibia, and humerus, he was estimated to be 164-170 cm tall. We used stature estimation from the Stature calculations formula for white males (taken from Trotter 1970). Radiography was obtained from the femur. It is noteworthy that there was platinum in his left femur, which was indicative of AM changes, fracture, and an operation.

Moreover, ossification was not complete (Figures 2, 2a and 2c). Osteoblasts have been active in bone formation, but osteoblast activity for calcification has not started yet, and bone modeling and remodeling are incomplete.

In the present study, the maximum length of the femur, five segmental measurements, and three proximal and two distal measurements were taken using anthropometric instruments such as an osteometric board and digital vernier caliper with a precision of 0.01 mm. The following parameters were measured according to the standard procedure suggested by Trotter and Glesser:

• Maximum femoral length (MFL): Distance from the most proximal point of the head of the femur to the most distal point of the medial condyle.

Figure 1. Excavation of skeletal remains scattered in Majnoon Island

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• Femoral neck length (FNL): The distance between the base of the head and the intertrochanteric line at the junction of the back of the neck with the shaft.

• Femoral neck circumference (FNC): Circumference of the neck at the middle of FNL.

• Intertrochanteric crest length (ICL): The most proximal point of the greater trochanter and the lowest point of the lesser trochanter.

• Medial condyle length (MCL): The linear distance between the most anterior and posterior points on the medial condyle.

• Lateral condyle length (LCL): The linear distance on the lateral condyle measured in an anteroposterior direction (Table 1).

Sample preparation for DNA analysis

The external surface of the bone is smoothed down to avoid any contaminants. The surfaces of bones were cleared from soil and mineral grains using a high-speed polishing system (metallic drill) (Balkan Universal 25,000 RPM Pendant Motor - TP884). The samples were successively washed in mild detergent, 5% bleach, sterile distilled water, 96% ethanol, and air-dried [21]. Thoroughly dried samples were pulverized using a sterilized blender (Qiagen Tissue Lyser II Sample Disruption Preparation Bead Mill in Walpole, MA, USA), then the powder was transferred to 5-mL sterile tubes.

DNA extraction

QIAamp® DNA Investigator Kit did the extraction with slight changes as the demineralization process was 200-300 mg of bone powder incubated in 15 mL of EDTA 0.5 M overnight at room temperature. The tubes were centrifuged for 5 min at 3000 rpm the next day, and the

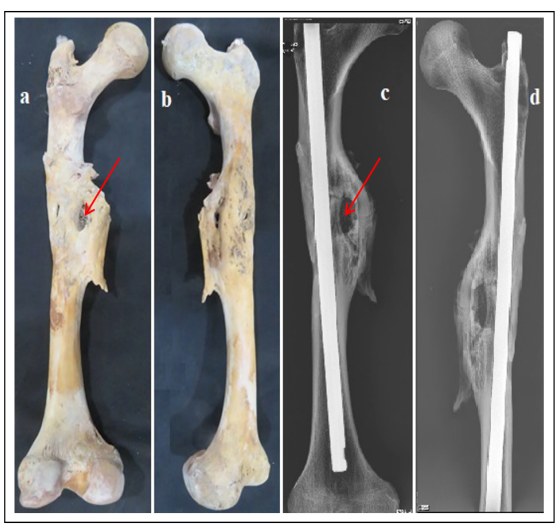

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Figure 2. a) Posterior view of the femur after operative treatment, b) Anterior/lateral view of the femur after operative treatment, c and d) X-ray images of an orthopedic implant

Table 1. Comparison of mean of MFL and other parameters

Parameters	Mean±SD	
	Right Side	Left Side
MFL	43.675±2.857	42.331±3.038
ICL	7.359±0.637	7.296±0.677
FNL	4.435±0.543	4.464±0.558
FNC	10.434±0.942	10.150±0.983
MCL	3.667±0.359	3.650±0.392
LCL	3.556±0.280	3.490±0.316

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supernatant was discarded. Then, 200 µL ATL buffer and 20 µL proteinase K were added to each sample in a sterile 15-mL test tube. The samples were then digested for three hours at 56 ˚C. After completing digestion, the extraction process was carried out according to the manufacturer›s instructions. We used FTA cards to collect blood samples from their participants. A tiny piece of blood-thin paper was inserted into the tube, washed for 5 minutes, and dissolved the buffer using 200 µL of buffering agent (repurification agent). Then, the sample was rinsed with water. The water was removed after 5 minutes (www.sigmaaldrich.com). After drying, the sample was ready for the PCR [22].

DNA quantification

The QC assays for template (DNA concentration) were built and set up with the assistance of the QIAgility automated DNA processing software (QIAGEN) and Quantifiler™ Trio DNA Quantification Kit (Life Technologies®. Foster City, CA) [23, 24]. The reaction was carried out in the AB 7500 Real-Time PCR System (Applied Biosystems) and HID real-time PCR analysis software, version 1.3, according to the manufacturer's instructions [25].

PCR amplification

Multiplex PCR was performed using the AmpFLSTR Minifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Two Multiplex PCR kits, including AmpFLSTR™ Yfiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) and Investigator Argus X-12 QS Kit (www.qiagen.com), were also used as complementary kits in some cases to ensure certainty.

The total volume of each reaction was 25 µL. According to the manufacturer's recommendations, the PCR amplification was done in SimpliAmp™ Thermal Cycler (Applied Biosystems). In samples with a small amount of DNA, the number of cycles was increased. Also, the AmpFLSTR Identifiler Direct PCR Amplification Kit was used for families' blood samples.

Statistical analysis

Samples were analyzed using GeneMapper ID software, [10, 26]. After obtaining genetic information for individuals and further analyzing the data and genetic software and genetic information banks, specific native software with the ability to search data and compare genetic and individual information is necessary [27, 28]. For the mentioned event with a high amount of genetic data, Noor Genetic Identification Software (NoorGIS), was used for genetic analysis and genetic computation, specially paternity index (PI) and combined paternity index (CPI) [17, 29]. Also, Familias software, was used for likelihood ratio analysis when the amount of genetic comparison was specified [30].

Table 2. Quantification tests' results

Mean Quantity ST (ng/ul)	Mean Quantity LT (ng/ul)	Mean Quantity YT (ng/ul)	IPC. Ct.	Degradation Index
0.117	0.074	0.112	27	1.6

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Table 4. The DNA analysis using the Investigator Argus X-12 QS kit

Sample Code	Family References	DXS10103	DXS8387	DXS10101	DXS10134	DXS10074	DXS7132	DXS10135	DXS7423	DXS10146	DXS10079	HPRTB	DXS10148
7538	Missing person	19	12	36	36	16	14	20	15	OL	20	13	28.1
F-8349	Mother	16-19	12	27-36	32-36	16-18	13-14	20-26	15-16	OL	17-20	12-13	24.1-28.1

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Genetic profiles were prepared for samples of families using the AmpFℓSTR Identifiler Direct PCR Amplification Kit. The sample used for this process included a piece of the femur. DNA quantitation results showed that the level of DNA in this sample was according to the Table 2. The Quantifiler™ Trio kit was used for this purpose, and we found large and small lengths of the degraded DNA and some DNA of the Y chromosome (Table 2). Accordingly, it was determined which PCR kit (Minifiler or Identifiler) should be used for each sample (Table 3). For this sample with a low DNA level, the number of PCR cycles was programmed in more than 30 cycles for the optimal profile. When the DNA with the minifiler kit was of inadequate quality, the kit attempted to analyze the standard sex chromosomes within relatives (Tables 3 and 4). The likelihood of paternity dependent in the STR

Table 3. The DNA analysis using Identifiler and Minifiler kits

		Identifier													
		Minifiler													
Sample Code	Family References	CSF1PO	D13S317	D16S539	FGA	D7S820	D18S51	D21S11	D2S1338	D19S433	D3S1358	D5S818	D8S1179	TH01	TPOX	vWA
7538	Missing person	12-13	11-12	11-12	19-24	8-11	17-18	28-29	17-20	14-14	16-17	-	13-13	8-9	11-12	16-17
F-8349	Mother	11-13	11-11	11-11	19-20	8-10	17-18	28-31.2	17-17	13-14	15-16	10-11	10-13	9-9	8-12	15-17
F-8350	Brother	12-13	11-12	11-11	19-24	8-11	18-18	29-31.2	17-20	13-14	16-18	11-14	13-13	6-9	11-12	17-18
F-8351	Sister	10-11	11-12	11-11	20-24	10-11	18-18	29-31.2	17-20	13-14	16-17	10-14	13-13	9-9	11-12	17-18

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Table 5. The DNA analysis using the Y-filer kit

Sample Code	Family References	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS635	DYS392	GATA_H4	DYS437	DYS438	DYS448
7538	Missing person	16	13	25	31	15	15	11-15	13	10	10	22	11	13	14	10	20
F-8350	Brother	16	13	25	31	15	15	11-15	13	10	10	22	11	13	14	10	20

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analysis was estimated at 1598029, and the probability of identification was estimated at 0.999999.

The likelihood ratio for Y filer STRs (Based on the YHRD database) was also calculated in 200041. Radiographic and genetic examinations ultimately confirmed that the dead body matched the relevant family (Table 5).

Discussion

DNA analysis showed that different autosomal STR and sex chromosome STR kits can be used for different qualities of DNA for a reliable response in the identifications. The quantification experiment determined that the sample concentration was >1 pg/µL. As a result, for valuable DNA, the Identifiler kit was used for 16 loci, and for a sample of LCN, the Minifiler kit was used for nine loci. Using Yfiler and Investigator Argus X-12 QS kits is essential when we know the identification was due to the presence and approval of one parent or sibling. Anthropometric and genetic examinations ultimately confirmed that the dead body matched the relevant family. However, due to the lack of medical records after 40 years, the evidence (operation of the femur as Ante Mortem) was confirmed by the missing person's family.

Conclusion

The present study showed that storing medical records as AM evidence and genetic examinations of missing persons can significantly determine missing persons' identities. In the future, all medical and dental records, patient samples (blood, pathology, teeth, etc.), and radiographic records should be stored in searchable databases to be used in possible accidents.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences (Code: IR.BMSU.REC.1398.066). Written informed consent was obtained from all participants.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to thank the International Committee of the Red Cross for their valuable contribution to this project.

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