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Forensic osteological investigations in Kosovo

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Abstract

A team of Finnish forensic experts performed investigations of alleged mass graves in Kosovo under the mandate of the European Union (EU). Human skeletal remains from two locations were examined. The remains contained three almost complete skeletons, and individual bones and bone fragments, part of which were burned. Injuries, pathological changes, and findings for identification purposes were examined and documented using standard methods of forensic pathology and osteology. Gunshot injuries were found in some cases, but reliable determination of the cause and manner of death was not possible. A discrepancy arose between the number of victims reported in information received from the presiding district court, and results of the investigations. The estimation of the minimum number of victims was mostly acquired by DNA analysis.

Keywords: Forensic science; Forensic osteology; Human remains; Human identification; DNA identification; Mass disaster; International co-operation

1. Introduction

During the armed ethnic conflict in Kosovo, in the Federal Republic of Yugoslavia (FRY), the European Union (EU) deployed a multidisciplinary forensic team to investigate alleged mass graves in the area [1]. A team of Finnish experts worked in Pristina under the mandate of the EU, and on the basis of a protocol on co-operation between the Institute for Forensic Medicine of Belgrade University, Yugoslavia, and the Department of Forensic Medicine, University of Helsinki, Finland. The EU Forensic Expert Team (EU-FET) was to investigate three alleged locations of ethnic Serbian victims and three alleged locations of ethnic Albanian victims. All locations represented a different number of victims, different time elapsed since death, and different information about events before and during the death of the victims. At the first stage, on 4–7 December 1998, EU-FET investigated skeletal remains reported to have originated from Volujak and Klecka. The aim of the work was to determine the cause and manner of death, estimate the time elapsed since death, and collect data for identification of victims. The remains had already been delivered to the Department of Forensic Medicine, University of Pristina. Local forensic experts had investigated the remains and assembled the skeletons from Volujak.

According to information received from the District Court of Pec, several Serbs, claimed to have been killed by ethnic Albanians in summer 1998, were found in Volujak on 3 October 1998. In Klecka, based on information received from the District Court of Pristina, 22 Serbs, including women and children, claimed to have been killed and cremated by ethnic Albanians in June–July 1998, were found on 27 August 1998.

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2. Materials and methods

The skeletal remains from Volujak contained three almost fully assembled adult human skeletons, and separate bones and bone fragments. The skeletons included all the long bones with the exception of one right humerus, one left ulna, and one right fibula. Two of the skeletons had a fragmented left radius, and one, a fragmented left ulna. One of the humeri was previously sawed and could therefore, only be measured approximately. Two skeletons included maxillae and a mandible. Hip bones were present in two of the skeletons.

The individual bone samples were of human origin. They contained one bone in 14 cases and 2–10 bones in 10 samples. Among the separate bone samples were one left humerus, two left radii, one right and one left ulna, and one right fibula. The samples also included some fragments of other long bones, two maxillary and three mandibular fragments.

The skeletal material from Volujak put together by the EU-FET is presented in Fig. 1. Remnants of clothing and other personal belongings were also present.

The skeletal remains from Klecka comprised 90 samples of human bone fragments, which were enclosed in separate pouches containing one or more bone fragments. Most of the pouches contained one fragment, 18 pouches had 2–10 fragments, and one pouch had 108 small, burned adult bone fragments. In total, 251 bone fragments were present. In all material, 181 (72%) of the bones were defined and 70 (28%) remained undetermined, of which 65 were in the pouch of 108 fragments. There were 57 vertebral fragments, 48 fragments of ribs, 18 cranial or mandibular fragments, and 52 other defined bone fragments.

No intact long bones were present which could be measured for stature estimation. Two of the samples contained hip bones and four samples included fragments of mandible. Three skull bone samples included the internal occipital protuberance.

Investigation of the human skeletal material by the EU-FET took place at the Institute of Forensic Medicine, University of Pristina. Specimens were extracted for DNA analysis, and the remains were left behind at the Institute after investigations were completed.

The remains were numbered according to the same grouping of samples used by the Yugoslavian investigators. Either assembled skeletons or separate bone fragments were counted as one sample. The samples from Volujak were identified with numbers 1001–1027, and the samples from Klecka, with numbers 2001–2090. The previously arranged anatomic order of the three skeletons from Volujak was accepted, for the most part, by the EU-FET.

Each skeleton and bone fragment was examined by two forensic pathologists and a physical anthropologist, except for the pouch containing 108 small fragments, which was investigated by only the physical anthropologist. The forensic pathologists described the preserved anatomic structure of the samples, and documented the sex characteristics, evidence of diseases and anomalies, and any injuries present on the bones. The investigation was performed using standard methods of forensic pathology and following the guidelines of the United Nations [2] and Interpol [3].

All equipment used by the EU-FET for X-ray, photography, and videotape documentation was brought from Finland. The equipment for medical and dental X-rays was the same one which was also used during the investigation of the victims from Racak [1]. An X-ray examination was performed on all investigated bones and X-rays were then examined by the forensic pathologists. In total, 70 X-rays were taken of the Volujak material and 95 of the Klecka material.

Further analysis was conducted of any foreign material present in X-ray examination. Samples were analyzed in the Crime Laboratory of the Finnish National Bureau of Investigation by using a scanning electron microscope (SEM) and performing an energy dispersive X-ray analysis (EDS).

The physical anthropologist confirmed bone and side determinations, and estimated sex, age, and stature. Sex estimation was accomplished by examining the form of the skull and pelvis and their sex characteristics [4], and performing anthropological measurements according to the Pearson method [5] with the modification by Stewart [6]. Age estimation was performed by examining the pubic bone using the methods of Suchey–Brooks [7] and Todd [8]. A plaster comparison model by France Casting was used with the Suchey–Brooks method. Furthermore, the sternal end of the ribs were examined [9]. For stature estimation the long bones were measured and the formula of Sjøvold [10] was used. Results were compared with the formulae and tables of Trotter and Gleser [11].

Maxillae and mandibles were investigated and the X-rays examined by the forensic odontologist, who documented postmortem dental status and recorded dental characteristics by filling the Interpol Disaster Victim Identification Form. Age estimation was conducted using the Johanson method [12]. There were a total of four dental X-rays of Volujak material and six of Klecka material. Dental samples were also photographed and videotaped.

The skeletons from Volujak and the bones from Klecka were all photographed separately from different directions. Gunshot injuries were also photographed showing the direction of the bullet. The total number of photographs was about 1600. In addition, videotape documentation was made, containing approximately 5 h of filmed material. Forensic investigators documented remnants of clothing and other material belongings that accompanied the skeletal remains from Volujak.

Specimens for DNA analysis were extracted from each skeleton from Volujak and each sample from Klecka; the total number of specimens was 38 and 93, respectively. DNA analysis was performed at the Department of Forensic Medicine, University of Helsinki between 20 February and 20 July 1999.

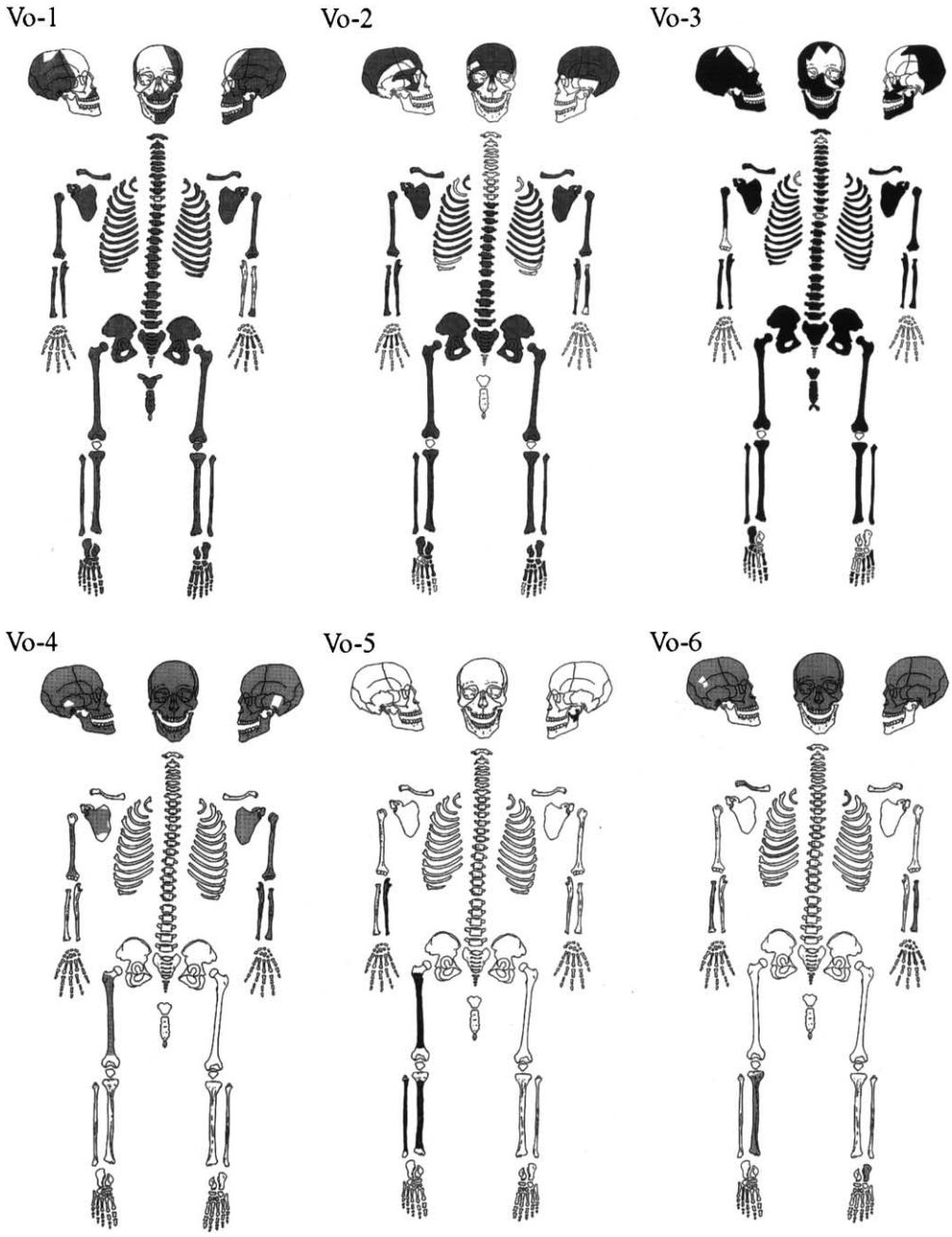

Fig. 1. Investigated skeletal material from Volujak.

Prior to DNA extraction, the bones were cleaned of dirt with sandpaper, after which they were crushed using a dental hand-drill or, in cases where pieces were too small to handle, they were smashed with a hammer, and the bone fractions were homogenized with a Mikro–Dismembrator U (B. Braun Biotech International). Approximately 1 g of bone powder from each sample was used for DNA extraction. The bone powder was mixed in extraction buffer (10 mM of TRIS pH 8, 100 mM of NaCl, 50 mM EDTA pH 8, 0.5% SDS) and samples were incubated with proteinase K (0.5 mg/ml) in water bath of +56°C from 4 to 24 h. After incubation, samples were centrifuged and the supernatant was used for standard phenol–chloroform extraction [13] with the aid of Phase Lock Gel (5′→3′ Inc.) tubes. Concentration of the extracted DNA was assessed using Centrex UF-2 30 kD tubes.

DNA was amplified with the AmpFlSTR Profiler PCR amplification-kit (PE Applied Biosystems) and analyzed with ABI Prism 310 capillary electrophoresis, according to manufacturer's recommendations. DNA profiles consisted of nine microsatellite markers [1] and sex was determined via the amelogenin locus. In all cases, DNA extraction and amplification were performed twice.

3. Results

3.1. Volujak material

Morphological investigation of the human skeletal material from Volujak revealed it to be the remains of a minimum of five adult male victims. Age was possible to estimate anthropologically in three cases and odontologically in one case. Degenerative changes were present on the bones of each victim. Stature was estimated anthropologically. The DNA results showed that the samples belonged to a minimum of five male victims. DNA profiles of nine bone fragments could not be reliably typed, and therefore, it was not possible to connect these to the other samples. There was no morphological objection to these remains being from the five aforementioned victims (Fig. 1).

Three of the victims had distinct gunshot injuries and one victim had a suspected gunshot injury. Also in the remains which were not connected by DNA analysis with the five victims were identified gunshot injuries. Gunshot injuries were present in four skulls, a lumbar vertebra in one case, a left elbow in another, and a right scapula and humerus in the third. Bullets or parts of bullets were not evident, with the exception of one skull, inside which was an almost intact bullet of an assault rifle (Fig. 2). Radiologically, foreign material was discovered in three victims, but this was shown to be of soil origin. The reason for fragmentation of some of the bones could not be reliably determined. The possibility that the fragmentation was the result of gunshot injuries could not be excluded. The remains of four of the victims and some of the unconnected bones had injuries, which were morphologically estimated to be postmortem damage inflicted by animals.

The bones and bone fragments were relatively heavy and greasy. The skeletal remains of all of the victims included

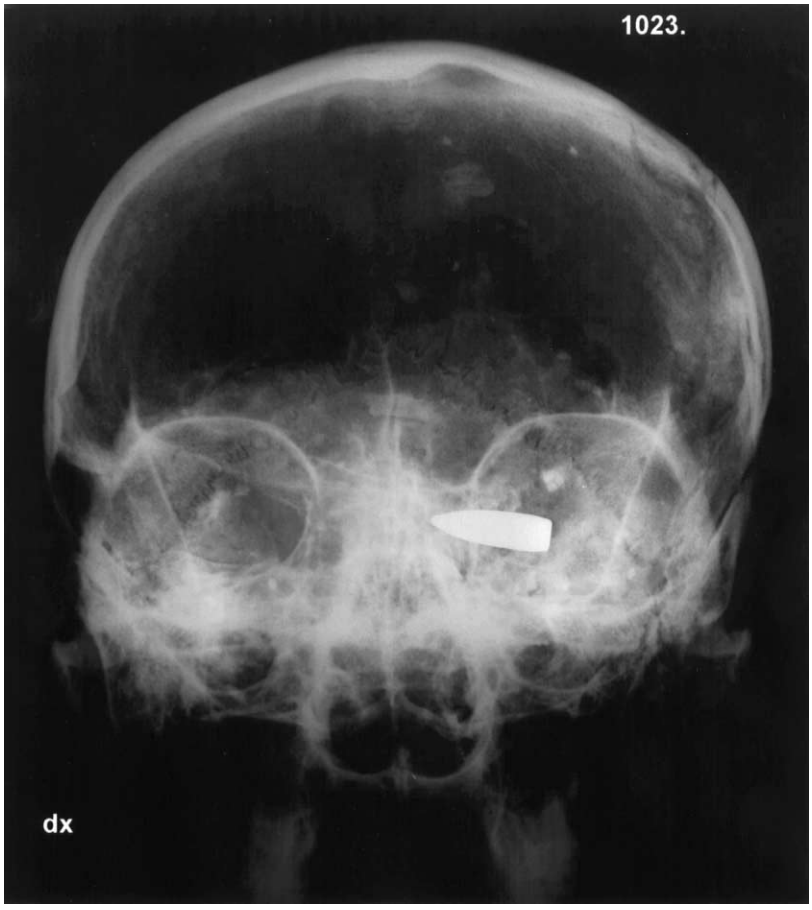

Fig. 2. X-ray of skull with a projectile inside.

Table 1
Main findings of the remains from Volujak

	Vo-1	Vo-2	Vo-3	Vo-4	Vo-5[a]	Vo-6[b]
Sex	M	M	M	M	M	M[c]
Age (years)	>45	>50	>50	67 ± 10[d]	–[e]	–[e]
Stature (cm)	170 ± 5	175 ± 5	177 ± 5	169 ± 5	178 ± 5	–
Degenerative changes	+	+	+	+	+	+
Soft tissue preserved	+	+	+	+	+	+
Gunshot injuries	+[f]	+	+	+	–	+
Remains of bullet	–	–	–	+	–	–
Other injuries	–	+[g]	+[g]	+[g]	–[h]	+[g]
Postmortem damage[i]	–	+	+	+	+	+

[a] Victims marked with codes.
[b] Samples not connected with the five victims.
[c] Estimated morphologically.
[d] Estimated odontologically.
[e] Morphologically estimated to be adult.
[f] Probable gunshot injury.
[g] Etiology of bone damage could not be specified.
[h] Disarticulation present.
[i] By wild animals.

some soft tissue remnants. The skull with the bullet inside also had 440 g of brain tissue remaining. Soft tissue was also found on another skull and on some other structures. The skeletal remains were covered with reddish soil material. Some bones had marks of mechanical cleaning.

The main findings of the remains from Volujak are presented in Table 1.

DNA analysis was performed on 38 samples from Volujak. For nine samples, DNA typing could not be reliably performed, and the remaining 29 samples consisted of five different profiles. All of these profiles were of male origin. Due to DNA degradation or DNA polymerase inhibition, not all of the profiles were complete for the 10 loci analyzed. D7S820 and CSF1PO were the two loci that were most commonly not reliably amplified. Results from these analyses are presented in Table 2.

3.2. Klecka material

The investigated separate bones and bone fragments from Klecka consisted morphologically of a minimum of three adult male victims. This conclusion was based on the

Table 2
Combined DNA profiles[a] from skeletal remains from Volujak and Klecka

Profiles	D3S1358	vWA	FGA	TH01	TPOX	CSF1PO	D5S818	D13S317	D7S820	Gender
Vo-1[b]	15–18	14–16	20–20	6–8	8–8	10–13	9–13	11–12	9–11	Male
Vo-2[c]	15–18	17–17	22–24	7–9.3	8–11	12–13	12–12	9–11	10–10	Male
Vo-3[d]	17–17	15–16	19–25	6–6	8–8	12–12	9–12	10–11	10–11	Male
Vo-4[e]	16–16	14–17	24–24	8–9	9–11	10–12	9–12	9–10	9–10	Male
Vo-5[f]	15–16	14–15	19–24	6–9	9–11	10–10	9–12	8–9	10–10	Male
Kl-1[g]	15–15	16–16	20–21	6–8	8–10	12–13	11–11	11–12	9–12	Male
Kl-2[h]	17–18	17–17	23–24	9–9.3	8–8	10–10	11–12	8–12	11–12	Male
Kl-3[i]	15–17	16–17	22–25	6–9	8–8	11–11	11–12	8–14	9–12	Male

[a] DNA profiles combined from all Volujak/Klecka samples. In cases where profiles were incomplete, the missing loci are listed below.
[b] Based on three samples, one complete profile. CSF1PO was missing twice.
[c] Based on four samples, three complete profiles. CSF1PO was missing once.
[d] Based on 10 samples, six complete profiles. D7S820 missing four times.
[e] Based on five samples, three complete profiles. TPOX missing once, CSF1PO twice and D7S820 twice.
[f] Based on seven samples, six complete profiles. FGA, TH01, TPOX, and D5S818 missing once.
[g] Based on 35 samples, 23 complete profiles. FGA missing three times, TPOX one, CSF1PO 10 times, D5S818 once, D13S317 six times, and D7S820 10 times.
[h] Based on 36 samples, 26 complete profiles. FGA missing twice, TH01 once, CSF1PO three times, D13S317 once, and D7S820 10 times.
[i] Based on 13 samples, 11 complete profiles. D7S820 missing twice.

Table 3
Main findings of the remains from Klecka

	Samples[a] ($n = 90$)	Victims[b] ($n = 3$)
Gunshot injuries[c]	1	1
Other injuries[c]	21	3
Signs of burning	49	3
Animal-inflicted damage	3	2
Soft tissue preserved	29	3

[a] Ninety samples containing different number of bones or bone fragments.
[b] Number of individuals was determined by DNA analysis.
[c] Nature of injury could not be specified.

number of pelvic structures and internal occipital protuberances. In one case age was estimated by anthropological examination. Another victim was odontologically estimated as middle-aged. Stature estimation could not be performed in the absence of intact long bones.

One sample, containing 108 small burned bone fragments, could not be connected by DNA analysis with the three victims because of the degree of fragmentation and burning of the bones. Osteologically, it was possible to determine 43 of the fragments. Morphologically, it was estimated that all fragments could be from one adult person.

In one of the samples, gunshot injuries were identified in the lumbar and sacral vertebrae. Altogether, 49 of the samples had signs of burning of variable severity. It was not possible to establish whether these were caused intravitally or postmortem. The etiology and intravital character of injuries present on 21 samples remained unclear. The reason or reasons for decomposition of the skeletons could not be specified. Postmortem damage evident in three samples was probably caused by animals. A small quantity of soft tissue was present on 29 samples.

The main findings of the remains from Klecka are presented in Table 3.

DNA was extracted from 93 samples; extraction was successful for 84 samples. These 84 samples comprised three different DNA profiles, all of which were for male individuals. Similar to the samples from Volujak, some of the typing resulted in partial DNA profiles, in most of which the loci D7S829 and CSF1PO were not reliably amplified. Results of these analyses are presented in Table 2.

4. Discussion

The aim of the investigations of the EU-FET was to conclude the cause, manner, and time of death of the victims of alleged mass graves, and to collect data for identification of the victims.

According to information received from the presiding district court, ''several'' persons were killed in Volujak and 22 persons including women and children were killed and cremated in Klecka. By using morphological, anthropological, odontological, and DNA analyses, the ''Volujak'' remains were shown to contain most likely the bones of five adult male victims, and the ''Klecka'' remains, those of three adult male victims. The chain of custody for the samples from the alleged finding places in Klecka and Volujak to Pristina could not be documented and verified by the EU-FET. Thus, the EU-FET was unable to confirm that all remains located in both areas were submitted for investigation, or that the remains were from the area from which they were purportedly collected. In addition, some of the investigated bone fragments could not be identified. Therefore, the possibility exists that among these fragments were the remains of a greater number of persons, possibly killed in Volujak, and the remains of another 19 persons, possibly killed in Klecka. Evaluation of the certainty of information received was beyond the possibilities of the EU-FET.

Gunshot injuries were identified in 3–4 cases and in one case from Volujak and Klecka, respectively. They were likely caused by assault rifle or another powerful weapon due to the destruction of substantial bones. A projectile was present inside one skull. The vitality of the injuries could not be reliably ascertained since these kinds of injuries can also be inflicted postmortem. If the gunshot injuries were inflicted before death, they would have caused the death of the victims because of injuries to the brain, abdominal aorta, and other vital organs. In investigation of victims from mass graves, the definition of cause of death often remains speculative or is even impossible [14].

The possible manners of death, according to the World Health Organization [15], in case the vitality of the gunshot injuries can be verified, are homicide, war or uncertain. Accident can also not be excluded.

The time elapsed since death was estimated as months or years. This conclusion was based on the physical appearance of skeletal remains and the presence of soft tissues, even brain tissue, in one of the skulls from Volujak. The rate of postmortem changes depends on many environmental factors. Determination of these details was also beyond the possibilities of the EU-FET.

The remains of four victims from Volujak and two victims from Klecka had injuries, which were morphologically estimated to be postmortem damage inflicted by animals. Many animals produce postmortem injuries to bones. The existence and manner of bone alteration depend on tooth morphology, jaw mechanism and strength of the animals, on clothing on the body, on amount of soft tissue, pre-existing wounds to the body, stage of decomposition, and on other factors [16–21].

The database for identification was collected during the investigation. The human remains from Volujak contained three almost fully assembled skeletons as well as individual bones and bone fragments. Morphologically, the remains were estimated to belong to five adult male victims. The stature of the victim was estimated in five cases, and the age,

in four. The minimum number of individuals was determined by DNA analysis. If necessary, the identity of the victims can be confirmed by comparing the obtained DNA profile with that of the putative relative.

The skeletal remains from Klecka comprised mostly burned fragments of bones, which could not be combined morphologically. Some of the fragments had male characteristics. The age of the victim was estimated in one case. No intact long bones were present for estimation of stature. Thus, the determination of minimum number of individuals and their identification were possible only by DNA analysis. The DNA profile is recorded for possible confirmation of victim identity.

Forensic investigations of mass disasters or mass graves are usually performed by multidisciplinary teams [22,23]. Forensic investigators and X-ray technicians are needed for the documentation. Proper X-ray facilities are also necessary since without them documentation, including odontological, is very difficult and will remain incomplete [24–26]. Forensic pathologists, odontologists, and physical anthropologists collect complementary data for identification. By using morphological and odontological methods, sex, age, and stature of the victims can be estimated, and possible signs of diseases and old traumas identified. These methods are relatively reliable, fast, and inexpensive for investigation of large numbers of victims. However, the possibilities of identification by using these methods are often limited. Antemortem data is frequently absent because it is destroyed during armed conflict or is unavailable for other reasons [27–29].

The DNA analysis was unsuccessful in nine cases for Volujak material and in nine cases for Klecka material. DNA degradation or DNA polymerase inhibition was the most likely explanation for unsuccessful amplification of some of the loci. In all 113 samples analyzed, three of the markers were always successfully typed. These were D3S1358, vWA, and amelogenin. TH01 was missing once, D5S818 twice, and TPOX three times. FGA and D13S317 were missing seven times. CSF1PO was missing in 17 profiles and D7S820 in 28 profiles. The marker most frequently missing D7S820 (absent in 32% of cases), is the second largest one, varying in size from 256 to 292 base pairs. Allelic dropout was also encountered in the amplification of samples. This was, however, noticed and corrected in re-amplification of the samples. The observed allelic dropout emphasizes the importance of careful analysis and interpretation of results of DNA profiling in such samples as presented here.

The experience of the EU-FET in Kosovo shows that DNA analysis provides a powerful methodology for identification of individuals and for estimation of the minimum number of individuals in mass disaster. DNA analysis is feasibly performed for a limited number of skeletal remains, but with an increasing number of skeletal samples, the corresponding time and cost of the work also increase. Moreover, in cases such as those presented here, the samples are often of poor quality, hampering the extraction of DNA and the subsequent amplification process. Thus, careful analysis and interpretation of DNA profiles are of the utmost importance in successful identification, where no discrepancy should remain concerning the findings obtained using various forensic methods.

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