Histological observations on adipocere in human remains buried for 21 years at the Tomašica grave-site in Bosnia and Herzegovina

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ABSTRACT

The Tomašica grave-site near Prijedor in the north of Bosnia is reported to be the largest primary mass grave discovered thus far relating to the 1992–95 war. A total of 275 complete bodies and 125 body parts were exhumed from it in 2013. Post mortem examinations of the victims showed that nearly all had died from gunshot injuries but an additional striking feature was the degree of preservation of many of the bodies, even 21 years on, with skin, soft tissues and internal organs still present in abundance and gross structures clearly identifiable. Histology was performed on 68 samples of soft tissue from a total 13 bodies, on both skin and internal organs, and the degree of preservation was assessed in terms of the ability to recognize microscopic structure. Further comparison was made with samples taken a month or so later (56 tissue samples from 9 bodies, all but one different from the first group), after the bodies had been covered in salt as a means of general preservation. Generally, at a microscopic level, skin and subcutaneous tissues were better preserved than internal organs, while tissues sampled at the time of autopsy were better preserved than those sampled weeks later.

KEY WORDS: Tomašica mass grave; adipocere; histopathology
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INTRODUCTION

The Tomašica mass grave near Prijedor in the north of Bosnia is reported to be the largest such finding in the country relating to the 1992–95 war. Most of the victims were men (and three women) from villages to the west of Prijedor, killed in July 1992 and their bodies buried within a few days at Tomašica, which was part of an iron ore mine [1]. The following year the grave was partially excavated ('robbed') and a large number of bodies were removed to Jakarina Kosa, and placed into what then became a secondary grave.

The Tomašica site was investigated three times from 2000 to 2006 with little found. The final exhumation from September to November 2013 eventually uncovered its full extent, with bodies buried up to 10 meters below the surface, in soil rich in clay and iron. The exhumations were carried out by the Bosnian Institute of Missing Persons assisted by the International Commission on Missing Persons (ICMP), and the subsequent autopsies (post-mortem examinations) were performed at the Šejkovača Center for Identification of Post Mortem Remains in Sanski Most.

The post-mortem examinations were carried out at the order of the Prosecutor's Office of Bosnia and Herzegovina, principally to identify injuries and cause of death as evidence of a potential war crime. At the same time, general and detailed observations were made for the purposes of identification (primarily by DNA) and any other evidence was recorded. Permission was given for tissue sampling as required.

A total of 400 sets of remains were recovered from the grave in this final exhumation, comprising 275 complete bodies and 125 body parts. Investigations showed that they represented at least 293 individuals, with an age range of 15–60+ years [2]. The vast majority died from gunshot injuries, mostly to the head, chest or both.

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Although the bodies had been buried for more than 21 years, a striking feature was the degree of tissue preservation in many of them. A substantial number, as expected, were completely or largely reduced to a skeleton, but in still more the skin, soft tissues and internal organs were well preserved, at least partially or completely. Body shape and integrity were maintained, deep muscle widely present, and facial features and tattoos occasionally identifiable. Even the detailed anatomy of the brain could be visualized in some cases (Figure 1).

Such soft tissue preservation here was attributed to the formation of adipocere (saponification), a variant of the normal decomposition process whereby body fats harden and preserve rather than liquefy. Its occurrence at Tomašica was presumably related to the depth of burial and the nature of the soil [3-5].

Adipocere is formed from body fat in a variety of contexts, including dry environments and water submersion. It is reported that anaerobic conditions, moisture and the presence of bacteria promote its formation [3]. In the context of mass graves, moisture often comes from the bodies themselves. The Tomašica bodies presented a form of hard brown adipocere with low surface moisture, more resembling mummification. With added ingrained soil material this made dissection all the more difficult. Additionally, on many of the bodies, the skin was heavily impregnated by blue crystals which were assumed to be Vivianite [6,7] and there was also extensive growth of mould, mainly on the surface but occasionally on internal organs as well (Figure 2).

Such large numbers of bodies with advanced adipocere formation provided an opportunity to study the tissues microscopically and to compare histological stains and, additionally, to compare preservation before and after the bodies had been covered in salt as a general preservative. (The bodies could not be reburied until the summer months. Because no refrigeration was available, the idea of liberally covering the remains in rough salt [NaCl] was adopted with successful results, not least in preventing growth of mould).

MATERIALS AND METHODS

Samples and histological preparation

The bodies at Tomašica had all been buried for the same 21-year time period, under the same general physical conditions. This allowed for a random choice of samples, targeting visually well preserved tissues from 21 bodies.

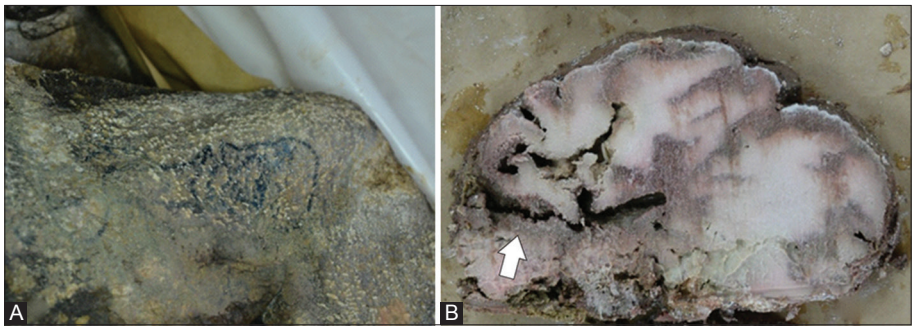

FIGURE 1. Features of preservation: (A) Clearly still visible tattoo on the back of the right forearm; (B) Well-preserved brain with an apparent still visible bullet track.

FIGURE 2. Body showing adipocere formation and a covering of mould, the latter developing post-exhumation. Blue staining (presumed to be from vivianite) is seen at the left wrist.

Two sample types were studied:

Group 1 - 68 samples of soft tissue from a total 13 bodies, obtained at the time of autopsy, of which 42 were from internal organs and 26 from skin and subcutaneous tissue. For the internal organs, heart and lungs were selected as being the best preserved, targeting the left ventricle and the upper lobes respectively.

Group 2 – 56 tissue samples from 9 bodies (all but one different from the first group), taken 1 month after completion of the autopsies and after the bodies had been immersed in salt for long-term preservation (two months). Of these, 18 were from internal organs and 38 from skin and subcutaneous tissue, the latter predominating here because of the damage to internal organs caused by the autopsy examination.

The samples were placed into formalin and routinely processed in the laboratory at the Department of Pathology, Medical Faculty, University of Sarajevo. Sectioning and staining of the Group 1 samples was performed in the UK at the Department of Forensic Medicine and Science, University of Glasgow, while the Group 2 samples were fully dealt with in Sarajevo. The study is approved by the Ethical Committee of the Faculty of Medicine, University of Sarajevo.

Staining

All slides were stained with hematoxylin and eosin (H&E), Masson's trichrome (MT), phosphotungstic acid-haematoxylin (PTAH) and Alizarin red (AR). The first two were aimed at determining structural preservation, the PTAH to look for fibrin and fungi, and the AR to try to identify any gunshot residues on the skin surface. The last two proved difficult to interpret and are not further analyzed in this paper.

Interpretation

All slides were examined by four experts: three forensic medicine specialists and one anatomic pathologist, all of which were blinded to the specific origin of the samples.

The total number of slides examined (H&E plus trichrome) was 136 for Group 1 and 112 for Group 2. The slides were examined by standard light microscopy on an Olympus BX43 microscope with built-in UC30 camera, images being captured using cellSens Standard software, version 1.8.1 [8].

Tissue preservation was graded as follows:

Grade 1 – nature of organ or tissue could not be identified.

Grade 2 – organ or tissue able to be identified but not any detailed structure.

Grade 3 – structures within organs or tissues able to be identified.

If examiners differed in their grading of a particular slide then the highest grade was used.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY) with the results presented as numbers and percentages. Differences between groups were tested using the Pearson's chi-square test and t-test (for mean values), a value of $p < 0.05$ being considered statistically significant.

RESULTS

Group 1

The grading of structural preservation in the Group 1 samples is presented in Table 1. Only one of the 26 skin and subcutaneous samples was given grade 2, i.e., recognizable as skin but with no visible structure, the other 96% being grade 3. Out of 42 samples of internal organs, 11 (26%) were grade 1, i.e., nature of tissue was not clearly identifiable, 9 (22%) were grade 2, and 22 (52%) were grade 3, having an identifiable histological structure. A statistically significant difference was found in the degree of structural preservation between the two types of sample (grade 1 $p = 0.0047$, grade 2 $p = 0.0484$, grade 3 $p = 0.0002$).

The average grade of tissue preservation was thus higher for the skin and subcutaneous samples where stratification into dermis, subcutaneous tissue and sometimes muscle was usually clear, but with no recognizable epidermis (Figure 3). Although overall the internal organs were less well preserved, bronchi and vessels were still clearly visible in lungs (Figure 4) and cross striations in myocytes in the heart (Figure 5).

In comparing H&E and MT staining of the same material, both were felt to be just as effective for skin and soft tissue, but in 9 of the 42 samples from internal organs MT was considered the better in demonstrating structures such as bronchi, vessels and myocytes, as illustrated in Figure 4.

Group 2

Results for the 56 tissue samples taken one month following completion of all autopsies and after the bodies had been preserved by salt for two months, are shown in Table 2.

Here, 32 out of 38 skin and subcutaneous samples (89%) were placed in grade 2 and only 6 (16%) were given grade 3. For the 18 organ samples, 10 (56%) were assessed as grade 1, i.e. not recognizable, and the other 8 (44%) as grade 2. Again, a

TABLE 1. Degree of structural preservation of Group 1 samples

Preservation	Skin and subcutaneous tissue		Internal organs	
	N	%	N	%
Grade 1	0	0	11	26.19
Grade 2	1	3.85	9	21.42
Grade 3	25	96.15	22	52.38
Total	26	100	42	100

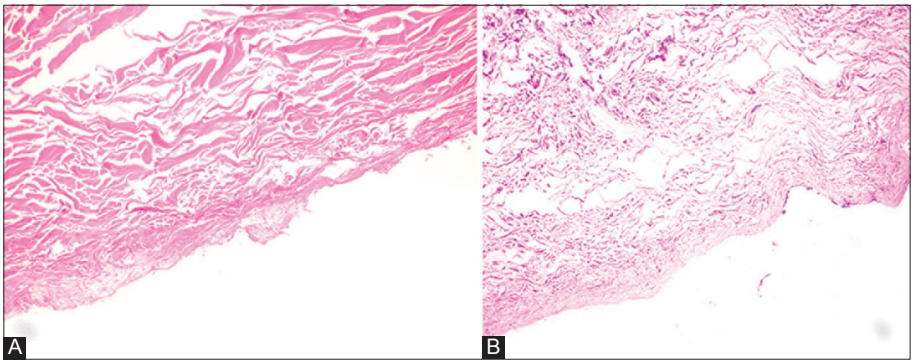

FIGURE 3. Surface of skin (hematoxylin and eosin [H&E], ×10) (A) section of Group 1 sample, (B) section of Group 2 sample.

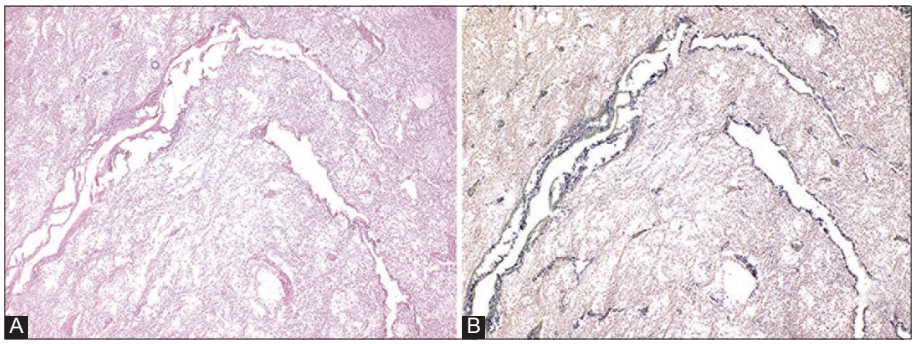

FIGURE 4. (A) Lung with poorly preserved alveoli (hematoxylin and eosin [H&E], ×10) and (B) same section stained for connective tissue (Masson's trichrome, ×10).

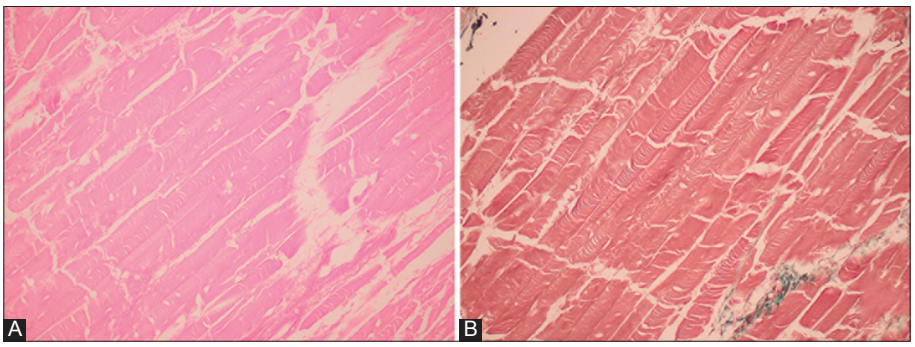

FIGURE 5. (A) Heart with relatively well preserved myocytes (hematoxylin and eosin [H&E], ×100) and (B) visible cross striations in myofibrils (Masson's trichrome, ×100).

statistically significant difference was found between the two sample types (grade 1 p = 0.0004, grade 2 p = 0.0327). The average grades of tissue preservation were significantly lower in Group 2 than those in Group 1, with 1.158 (±0.3695) for skin samples and 0.44 (±0.5113) for internal organs, $p < 0.001$).

Comparison of average grades between Group 1 and 2 is presented in Table 3.

DISCUSSION

In forensic practice, exhumations are usually carried out to establish identity or clarify cause of death although, depending on the circumstances, neither may be achieved [9,10].

Examination of remains from mass graves has particular challenges because of the numbers of bodies involved, the methods of deposition, the nature of the burial site, the length of time the remains may have been in the grave and the potential for body disruption. All of these applied at the Tomašica site near Prijedor, but much could still be achieved to assist towards both identification and criminal prosecution.

This study of the Tomašica remains focusses only on one small part of the overall investigation, related neither to identification nor cause of death, but to observations on the striking degree of preservation of many of the bodies, specifically to microscopic appearances of preserved soft tissues and recognition of detailed structure.

Histopathological examination of exhumed human remains has been reported previously. Karger at al. [11] investigated 155 consecutive exhumation cases with post-mortem intervals ranging from 8 days to 8 years, noting that in 103 cases in which the cause of death was able to be determined histology was considered decisive in 29% cases. In the examination

TABLE 2. Degree of structural preservation of Group 2 samples

Preservation	Skin and subcutaneous tissue		Internal organs	
	N	%	N	%
Grade 1	0	0	10	55.55
Grade 2	32	84.21	8	44.45
Grade 3	6	15.79	0	0
Total	38	100	18	100

TABLE 3. Comparison of average grades of preservation between Groups 1 and 2

Samples	Number	Average grade	*p*
Group 1	68	1.529±0.7624	<0.001
Group 2	56	0.929±0.5345	

of 83 bodies inhumed in airtight lead coffins for more than 150 years Green [12] performed histology on selected cases using H&E, MT and reticulin stains and identified micronodular cirrhosis in the liver and areas of fibrosis in the lungs suggestive of old tuberculosis and a lung abscess. Szleszkowski et al. [13] examined the results of 371 exhumations performed over a 31-year-period and reported positive histopathological findings in some cases, albeit the average number of days from burial, i.e. 74, was relatively short.

Numerous studies focus on the formation of adipocere and the chemical processes behind it [3,5,14-16] but few have looked at its degradation after exhumation. Frund and Schoenen [17] investigated the rate of degradation of five different samples under laboratory conditions, calculating a half-life of 293 days. They found that adipocere degraded in less than 10 years when exposed to air and soil microbiota, with the lowest half-life being for adipocerous material buried in biologically active field soil (mean of 1.5 years). They stressed the influence of air, moisture and fungal growth in accelerating the degradation of adipocere. In another laboratory study, Pfeiffer et al. [18] found that degradation of adipocere was most affected by the presence of gram positive bacteria, while Fiedler et al. [19] noted that it was also influenced by worms and fungal enzymes.

Of 400 sets of remains, buried in a mass grave for 21 years, soft tissue preservation was at times remarkable. Tissues were sampled from a random group of well-preserved bodies, both skin and internal organs, and the degree of preservation was assessed and graded microscopically. A further group of bodies (all but one different from the first group) was sampled several weeks later, after they had been encased in salt as a general preservation measure, and the microscopic preservation was compared with that of the first group.

In both groups it was shown that skin and subcutaneous tissue was better preserved than internal organs in terms of recognizable histological structure. It was further shown that overall preservation was better in the autopsy group than in those later preserved in salt, indicating continuing breakdown at least at a microscopic level. In only 5 out of 56 histology sections (9%) in Group 2 could any detailed structure be recognized, all from the skin samples and none from the internal organs. The comparable figure from the Group 1 samples was 47 out of 68 (69%).

Sections were stained with H&E and MT which appeared to work equally well on the skin and subcutaneous tissues in both groups and on the internal organs of Group 2. The only difference was for the internal organs of Group 1 where trichrome was judged superior in demonstrating histological structure in 9 of the 42 sections.

The more rapid decomposition of internal organs is most likely due to their higher moisture and lower lipid content, while the rich mineral soil at Tomašica (an old iron ore mine) being in contact with the skin, may have influenced the formation of fatty acid salts and thus promoted the adipocere formation.

CONCLUSION

At the microscopic level, degradation of adipocere continues – or more likely accelerates – after exhumation from a combination of exposure to air, handling, dissection and the enzymatic activity of microbiota. In terms of recognizable histological structure, skin and subcutaneous tissue was better than internal organs while, not surprisingly, the average grade of preservation overall was significantly higher in tissues sampled at the time of autopsy than in those taken a month or more later. In the absence of refrigeration, the method employed to preserve bodies after autopsy, by covering them with ordinary salt (NaCl), proved reasonably effective, not least in lowering moisture content and preventing further growth of mould, but did not halt tissue degradation at a microscopic level.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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