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Technical note

Technical note: Improvement of cadaveric skin samples (with severe morphological alteration connected to putrefaction or injury) by an extended histological processing

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ABSTRACT

Introduction: The microscopic study and the interpretation of skin samples with advanced post-mortal phenomena or with particular destructive injuries is problematic for the forensic pathologist. In an attempt of restoring the histological architecture of cadaveric skin and overcoming these types of problem, the Authors performed a histological processing that was longer than the standard: it was extended until 62 days to evaluate the improvement of the microscopic morphological aspect.
Materials and methods: Cutaneous samples were taken from 25 cadavers (5 typologies of skin: charred, putrified, corifed, mummified and partially skeletonized), fixed with a 10%-buffered formalin and then processed in two different ways: one half of the samples was routinely addressed to the standard-time automatic technique, while the other half was manually processed with prolonged times. All the slides were then stained in Hematoxylin–Eosin.
Results: The standard-processed slides demonstrated marked morphological alterations and artefacts at the microscopic observation; conversely, those processed with the prolonged manual technique showed an improvement in the morphological structure, sometimes permitting the identification of the anatomical components.
Conclusion: Though it is characterized by the inconvenience of protracted times, the application of a long-term manual histological processing to cadaveric skin samples with advanced post-mortal alteration permits to better observe the anatomical architecture of skin and it could be useful and helpful in the evaluation of such cases.

1. Introduction

The most important aims of the autopsy on fresh cadavers and of the histopatological analysis is to detect the cause and the manner of death. Sometimes, a cadaver could be exhumed for a second autopsy, due to various necessities (verification of data collected at previous autopsy [1,2], historical interest [3], supposed medical malpractice [4], personal identification [5], the suspect of a criminal death [6], and in particular due to poisoning [3,4,7] or other typology of homicide [4,8]).

Furthermore, whenever autopsy had not been performed and doubts concerning the cause of death still exists, the exhumation represents an even more important tool for the investigation of the circumstances of death [9]. Thus, the necessity of the execution of macroscopic and microscopic analysis on exhumed or deeply modified anatomical structures implies that the forensic pathologist always explores new methodologies in the preparation of the histological slides to improve their final quality: in this perspective, a series of factor [10] (like the experience of the pathologist and his/her subjectivity) plays an important role.

The standard preparation of the biological samples (histological processing) consists in three phases [11]: the progressive dehydration by a series of passages in ethanol with subsequent growing concentration; the diaphonization, (a series of transits in a paraffin's solvent, that substitutes xylene and makes the sample clear due to the complete loss of the water in the tissue); the final inclusion in paraffin. The purpose of this methodology is the transformation of the different tissues' compactness into a

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Table 1
Data concerning the population of the study.

Type	Case #	Lesivity	Sex	Age	Preservation	Cause of death	Sample site
Group 1	1	Homicide thermal	F	41	Charred	MOF	Torax
Charred	2	Homicide thermal	M	66	Charred	MOF	Torax
	3	Homicide thermal	M	26	Charred	Burns	Torax
	4	Suicide thermal	M	38	Charred	CO intoxication	Torax
	5	Traffic accident thermal	F	19	Charred	Burns + septic shock	Torax
	6	Accidental thermal	M	35	Charred	MOF	Leg and foot
	7	Accidental thermal	F	44	Charred	CO intoxication	Torax
GROUP 2	8	Blunt homicide	F	38	Exhumed after 3 yr	Cranial-encefalic injuries	Torax, arm and shoulder
Putrified	9	Blunt homicide	F	31	Putrified	LSV	Scalp, torax and leg
	10	Gunshot homicide	M	35	Putrified	Single Gunshot causing cranial-encefalic injuries	Scalp, back, arm and leg
	11	Strangling homicide	F	33	Exhumed after 4 yr	Strangling	Neck and arm
	12	Natural death	M	58	Exhumed after 3 months	Bronchopneumonia	Face, torax and leg
	13	Accidental thermal	F	32	Putrified	Burns	Scalp, shoulder and abdomen
	14	Hanging	M	41	Putrified	Mechanical asphyxia by hanging	Neck
GROUP 3	17	Natural death	M	70	Found after 1 month	Stroke	Abdomen, tigths and third finger of both hands
Corified	18	Natural death	M	66	Found after 2 months	Stroke	First and second finger left hand (base)
	19	Natural death	F	59	Found after 4 months	Not determined	Tight, calf, knee, left heel and right foot
	20	Natural death	F	57	Exhumed after 11 yr corified	Not determined	Abdomen and left hand
	21	Hanging	M	35	Corified groove	Mechanical asphyxia by hanging	Neck
	22	Hanging	F	28	Corified groove	Mechanical asphyxia by hanging	Neck
	23	Hanging	M	33	Corified groove	Mechanical asphyxia by hanging	Neck
GROUP 4	15	Natural death	M	37	Found after 5 yr	Not determined	Leg and foot
Mummified	16	Natural death	F	79	Found after 15 yr	Not determined	Back, arm and gluteus
GROUP 5	24	Natural death	M	NAS*	Found after 9 yr	Not determined	Skin
Skeletonized	25	Natural death	M	77	Exhumed after 11 yr	Not determined	Scalp, pubis and malleolus

* NAS meaning a not identified victim.

homogeneous and uniform solidity for the cutting process [12]. Though the utilization of these procedures, sometimes the result of the histological preparation is not optimal. To overcome this difficulty and improve the quality of the preparation, in addition to using rehydration solutions such as Sandison's solution [13], the Authors present their experience with an alternative preparation of biological sample consisting in a long-term manual preparation of skin samples with severe morphological alteration connected with post-mortal changes or fire.

2. Material and methods

A total number of 25 cadavers (11 female and 14 male individuals) were included in the study and subdivided in 5 groups: charred, putrefied, corifed, mummified and partially skeletonised cadavers. Age range was between 19 and 79 years old, all the corpses underwent to a judicial autopsy at the Section of Legal Medicine (University of Milan) in the period 2012–2014 and only 1 cadaver was not identified. The seven victims belonging to the first group were charred and all died in relationship with fire events (2 homicides, 2 suicides, and 3 accidental events). The second group was composed by individuals showing advanced putrefaction: 4 victims of homicide (2 blunt trauma, 1 gunshot and 1 manual strangulation), 1 natural death and 1 accidental death. In the third group, 7 corified cadavers were considered: 2 died for natural causes and 3 committed suicide by hanging; for the other 2 subjects (1 cadaver found at home 2 months after the alleged death and an exhumed cadaver recovered after 11 years in a zinc coffin) the cause of death was not determined due to the severe grade of transformation of the tissue. The 2 victims of the fourth group were found mummified after a long period (respectively, about 5 and 15 years after the alleged death). Finally, the last 2 individuals were partially skeletonised, and the cause of death was presumably attributed to natural events due to the absence of signs suggesting lesions (Table 1).

Skin was taken in a lozenge shape (with dimensions of 0.5 cm × 2.0 cm × 2.0 cm) from different anatomical regions: head (frontal, temporal, parietal, malar region), neck (anterior cervical region), thorax (mammary and dorsal region), upper limbs (arm and elbow), lower limbs. For each anatomical region, 2 different samples were taken. After the fixation in 10%-buffered formalin, all the biological samples were processed in two different ways. One half of the samples was subjected to routine standard-time automatic technique with alcohol dehydration, diaphonization and paraffin embedding by an automatic *Histo-Line Laboratories—ATP 700*, in about 20 h. The procedure included: 2 passages in 95% ethanol, 4 transits in absolute ethanol, 3 passages in a substitute of

Table 2
Procedures and timing of the automatic processing.

	Automatic processing
Dehydration	
1st alcohol 96%	1 h
2nd alcohol 96%	2 h
1st alcohol 99%	1 h
2nd alcohol 99%	1 h
3rd alcohol 99%	1 h
4th alcohol 99%	2 h
Diaphonization	
1st xilene's substitute	1 h
2nd xilene's substitute	1 h
3rd xilene's substitute	2 h
Inclusion	
1st paraffin 60 °C	2 h and 30 min
2nd paraffin 60 °C	2 h and 30 min
3rd paraffin 60 °C	2 h and 30 min