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RESEARCH ARTICLE

Characterization Of Changes in The Soft Tissues of The Wound Canal in An Intravital Gunshot Wound Using Morphometric and Immunohistochemical Methods

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ABSTRACT

Forensic examination of a gunshot injury is considered one of the most difficult types of forensic examinations. The problem of establishing the lifetime and duration of gunshot injuries of soft tissues remains among the topical in forensic medicine. In this article, we have studied a gunshot wound using morphometric and immunohistochemical methods.

KEYWORDS:
gunshot injuries, forensic medical examination, wound canal, immunohistochemical methods, lifetime, prescription.

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INTRODUCTION

Forensic examination of a gunshot injury is considered one of the most difficult types of forensic examinations. Firearms in the process of their use have specific features. In the process of producing an examination related to gunshot injuries (hereinafter referred to as GI), a forensic expert must solve a set of issues of a special nature. The problem of establishing the lifetime and duration of gunshot injuries of soft tissues remains among the topical in forensic medicine. As is known, classical histological methods serve as the basis for expert assessment of the duration of gunshot injuries, although their accuracy and heuristic value, alas, have already been exhausted in terms of development. Modern immunohistochemical methods (hereinafter IHC) can provide significant assistance in clarifying the duration of the gunshot injury. To achieve this goal and obtain maximum information about the object under study in each specific case, it is necessary to use a set of instrumental and laboratory research methods [1,3,4,5].

Aim of the study

To characterize the gunshot wound using morphometric and immunohistochemical methods.

MATERIALS AND RESEARCH METHODS

In the course of the expert activity, soft tissues were examined along the wound channel with the capture of the zone of necrosis and molecular concussion in corpses that died from GI both at the place of detection and with some experience. In the wall of the wound channel in the zone of necrosis, there is a loss of thin structures that were barely noticeable. In the zone of molecular concussion, the histological picture remains more intact. Small areas of necrosis with turbid cytoplasm and pictonic or decaying nuclei are traced.

The resulting tissue fragments were subjected to standard paraffin wiring and a number of histological and histochemical stains were used. Further, part of the material was subjected to standard IHC studies using an indirect immunoperoxidase method of study using a panel of antibodies to fibrinogen, smooth muscle cell actin, vimentin, sarcomeric actin, fibronectin, CD antigens, IH - lambda on immunosuppressors [5,6,7,9]. The techniques used are presented in Table 1.

Table 1: Used histological and IHC techniques

Methodology	Number of objects
Staining with hematoxylin and eosin	38
IHC reaction with antibodies to vimentin and to actin of smooth muscle cells	12
IHC reaction with antibodies to fibrinogen	28
IHC reaction with antibodies to fibronectin	6
IHC reaction with antibodies to sarcomeric actin	13
IHC with antibodies CD -68	6
IHC with antibodies to IH - lambda	10

Morphometric analysis was carried out using a micrometer object with a magnification of 10x20, the diameter of the field of view is 880 μm, and at 10x40, the diameter of the field of view is 460 μm.

The circumstances of the case and the relevant expert materials have been studied in detail.

As a comparison group, we used material obtained from a stab wound in the same period of death, which amounted to 3 observations among males in the age aspect from 24 to 68 years.

RESEARCH RESULTS

Based on Table № 2, this material was 16 cases and was distributed by gender, age, location of damage, type of thanatogenesis, type of gunshot injury (bullet and shot wounds), shot distance, according to the degree of alcohol intoxication.

Table 2: Characteristics of the study material (lifetime gunshot injuries)

№	gender	age	localization of injury	type of thanatogenesis	the duration of the experience of the trauma	type of gunshot injury	shot distance	presence of alcohol (0%)
1	m	52	head	cerebral	> 30 minutes	shot	in the field of action of additional factors of the shot	no
2	m	67	breast	heart + pulmonary	15-30 minutes	shot	point blank	no
3	m	31	breast	heart + pulmonary	15-30 minutes	shot	point blank	no
4	m	30	breast + stomach	cerebral + heart + pulmonary	> 30 minutes	shot	in the field of action of additional factors of the shot	k-3,81, m-5,29
5	m	36	breast + limbs	pulmonary	15-30 minutes	shot	point blank	k-2,26
6	m	51	breast	heart + pulmonary	>30 minutes	shot	in the field of action of additional factors of the shot	no
7	m	58	head	cerebral	15-30 minutes	bullet	point blank	k-1,55
8	w	46	head + breast + stomach + limbs	cerebral + heart + pulmonary	>30 minutes	bullet	in the field of action of additional factors of the shot	no
9	m	48	breast +	cerebral +	>30 minutes	bullet	in the field of	no

			limbs	heart + pulmonary			action of additional factors of the shot	
10	m	66	breast	heart + pulmonary	>30 minutes	bullet	in the field of action of additional factors of the shot	k-2,95, m-5,32
11	m	36	head + breast + limbs	cerebral + heart + pulmonary	15-30 minutes	bullet	in the field of action of additional factors of the shot	no
12	m	27	head + breast + stomach + limbs	cerebral + heart	15-30 minutes	bullet	in the field of action of additional factors of the shot	no
13	m	18	breast + stomach	heart + pulmonary	15-30 minutes	bullet	in the field of action of additional factors of the shot	no
14	m	23	head + breast + stomach	cerebral + pulmonary	15-30 minutes	bullet	in the field of action of additional factors of the shot	no
15	m	21	breast + limbs	heart + pulmonary	15-30 minutes	bullet	in the field of action of additional factors of the shot	no
16	m	54	breast	Pulmonary-cerebral	8 days	bullet	point blank	no

The average age of the victims varied in the age period from 18 to 67 years, men dominated, which amounted to 15 observations. Non-fatal alcoholism was noted in 4 cases. Isolated trauma was observed in 7 cases, combined - in 9.

In the course of thanatogenetic analysis, it was revealed that the combined type of thanatogenesis prevailed in 13 cases, the isolated type - in 3 cases.

In the course of the work, the IHC method was adapted to the forensic material. For this, paraffin blocks with the material are re-poured into special cassettes and subjected to wiring through an immunostable. The use of classical histological stains gave a picture of the classical GI, slightly different from that described in the literature [10,11,12,13].

As a result of the IHC reactions, it was revealed that high expression of fibrinogen is observed in the intravital GI, up to the zone of molecular shock.

At the same time, in the intravital GI, there is a focal loss of expression of sarcomeric actin in the zone of the canal muscles.

We also compared changes in the cytoskeleton of stromal and vascular cells. Lumpy disintegration of vimentin and actin of smooth muscle cells was noted.

There is a negative immunoreactivity of the zone of thermal necrosis in the canal wall. Diagnostically significant differences were revealed in the form of disorganization of the cytoskeleton structure in the cells of the vessels and stroma of the canal wall.

The use of other staining techniques did not give such a pronounced difference.

When using antibodies to fibronectin, the differences between intravital GIs were insignificant (only focal intravenular expression).

In general, we can say that we have investigated the possibility of using plasma substances and proteins of the cytoskeleton for the diagnosis of GI in vivo.

In addition, the method of establishing the rate of dying was applied, which in our observations revealed a lightning-fast as well as a fast rate of death.

Concerning the establishment of the limitation period for the experience of a gunshot injury. The IHC picture of soft tissue injuries was analyzed in observations of intravital gunshot injuries in observations with the experience of trauma. Markers of the lifetime of such injuries have been established. The absence of an early leukocyte reaction in the first hours after injury, excluding tissue intravascular leukocytosis, was shown. This feature continued in the future.

A morphometric method for establishing the duration of injuries in case of a gunshot injury has been tested using layer-by-layer histostereometry of zones in the depth of the canal.

When comparing the indices of the results of histostereometry in the depth of the wound canals of the same period, we note a slowdown in the inflammatory response with a gunshot and an acceleration in stab and cut wounds at the initial period of experience up to 30 minutes, with a period of about 7 days we noted an equalization of

indicators [8]. The results are shown in the table № 3.

DISCUSSION OF RESEARCH RESULTS

Classical methods of histological examination do not always allow to reliably establish the lifetime of GI, which finds its literary confirmation [2].

At the same time, the exudation of fibrinogen as a glycoprotein of acute inflammation is explained by the reaction to acute hemodynamic disturbance with an early release of plasma substances into the tissue.

Loss of expression of sarcomeric actin indicates the destruction of the contractile cytoskeleton in the GI zone. This is evidenced by the lumpy disintegration of vimentin and myosin of smooth muscles in the stroma and vessels in the zone of molecular concussion during GI.

A negative reaction in the zone of thermal necrosis of the wound canal is due to the coagulation of proteins.

As a result of a study based on the immunohistochemical study of the soft tissues of the wound canal wall in GI, the use of antibodies to fibrinogen and cytoskeletal proteins is shown to be promising for establishing the lifetime of such injuries.

When comparing gunshot and stab injuries, the differences relate mainly to changes in the cytoskeleton of cells. With GI, these changes are much more pronounced than in the comparison group and the control group.

Thus, the problem of establishing the lifetime of GI can be considered solved.

The deceleration of the leukocyte reaction in the zone of molecular shock requires further study using morphometric methods. Namely, it is advisable to assess the density of the infiltrate in the depth of the wound channel, taking into account its composition.

The pathology of muscle fibers can be explained by thermal and mechanical destruction of cell membranes with damage to the contractile apparatus and infiltration of the cytoplasm with fiery substances.

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Table 3: Comparison of the results of morphometry of firearms and stab and cut injuries

Necrosis	3±3,4 m=0,4	1±0,4 m=0,1
Zone of reactive changes / Zone of molecular shock	2±1,5 m=0,2	2±0,7 m=0,2
The area of the lesion, the duration of the experience of the trauma up to 30 minutes		
Necrosis	1±0,1 m=0	1±0,1 m=0
Zone of reactive changes / Zone of molecular shock	1±0,1 m=0	1±0,1 m=0
Lymphocytes, the duration of the experience of trauma is about 3 days		
Necrosis	25±7,5 m=2,4	13±3,3 m=1,0
Zone of reactive changes / Zone of molecular shock	23±3,3 m=1,0	11±2,4 m=0,8
Neutrophils, trauma experience about 3 days		
Necrosis	10±2,8 m=0,9	18±4,7 m=1,5
Zone of reactive changes / Zone of molecular shock	8±2,1 m=0,7	12±3,0 m=1,0
Macrophages, the duration of the experience of trauma is about 3 days		
Necrosis	10±3,1 m=1,0	7±1,9 m=0,6
Zone of reactive changes / Zone of molecular shock	9±2,8 m=0,9	6±1,8 m=0,6
The area of the lesion, the duration of the experience of the trauma is about 3 days		
Necrosis	1±0,1 m=0	1±0,1 m=0,1
Zone of reactive changes / Zone of molecular shock	1±0,1 m=0	1±0,2 m=0,1
Lymphocytes, the duration of the experience of trauma is about 5 days		
Necrosis	25±6,5 m=2,1	11±3,2 m=1
Zone of reactive changes / Zone of molecular shock	20±2,4 m=0,8	9±2,7 m=0,8
Neutrophils, the duration of the experience of trauma is about 5 days		
Necrosis	9±2,3 m=0,7	16±3,7 m=1,2
Zone of reactive changes / Zone of molecular shock	8±1,7 m=0,5	10±3,4 m=1,1
Macrophages, the duration of the experience of trauma is about 5 days		
Necrosis	10±2,4 m=0,8	8±1,9 m=0,6
Zone of reactive changes / Zone of molecular shock	8±1,9 m=0,6	7±1,5 m=0,5
The area of the lesion, the duration of the experience of the trauma is about 5 days		
Necrosis	1±0,1 m=0	1±0,2 m=0,1
Zone of reactive changes / Zone of molecular shock	1±0,1 m=0	1±0,2 m=0,1
Lymphocytes, the duration of the experience of trauma is about 7 days		
Necrosis	12±3,1 m=1,0	13±3,1 m=1,0
Zone of reactive changes / Zone of molecular shock	9±2,1 m=0,7	10±2,1 m=0,7
Neutrophils, trauma experience about 7 days		
Necrosis	6±1,9 m=0,6	6±2,0 m=0,6
Zone of reactive changes / Zone of molecular shock	3±1,6 m=0,4	3±1,5 m=0,3
Macrophages, the duration of the experience of trauma is about 7 days		
Necrosis	13±2,7 m=0,8	15±2,7 m=0,8
Zone of reactive changes / Zone of molecular shock	11±1,8 m=0,6	11±1,8 m=0,6
The area of the lesion, the duration of the experience of the trauma is about 7 days		
Necrosis	1±0,1 m=0	1±0,1 m=0
Zone of reactive changes / Zone of molecular shock	1±0,2 m=0,1	1±0,2 m=0,1
Lymphocytes, the duration of the experience of trauma is about 10 days		
Necrosis	7±1,5 m=0,5	10±2,7 m=0,9
Zone of reactive changes / Zone of molecular shock	7±2,2 m=0,7	12±2,0 m=0,6
Neutrophils, trauma experience about 10 days		
Necrosis	5±2,0 m=0,6	6±1,8 m=0,9
Zone of reactive changes / Zone of molecular shock	2±1,1 m=0,3	4±1,5 m=0,5
Macrophages, trauma experience about 10 days		
Necrosis	14±2,5 m=0,8	15±2,5 m=0,8

Zone of reactive changes / Zone of molecular shock	12±2,3 m=0,7	15±3,2 m=1,0
The area of the lesion, the duration of the experience of the trauma is about 10 days		
Necrosis	1±0,2 m=0	1±0,2 m=0
Zone of reactive changes / Zone of molecular shock	1±0,2 m=0	1±0,1 m=0