Legal Medicine 14 (2012) 1–5

Contents lists available at SciVerse ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Renal immunohistochemical investigation for the differentiation of the cause of multiple trauma fatalities

Tomoko Sugimura [a], Elaine Lu Wang [b], Masayuki Kashiwagi [a], Kenji Hara [a], Aya Matsusue [a], Brian Waters [a], Shin-ichi Kubo [a,*]

[a] Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, Japan
[b] Department of Legal Medicine, Kanazawa Medical University, Japan

ARTICLE INFO

Article history:
Received 6 June 2011
Received in revised form 15 September 2011
Accepted 15 September 2011
Available online 13 October 2011

Keywords:
L-FABP
SP-A
Multi-trauma fatality
Kidney
Immunohistochemistry

ABSTRACT

In fatalities with multiple traumatic injuries, it is important to determine the severity of trauma, the main damaged organ, and the antemortem pathophysiological condition. We examined 63 cases within 48 h of the postmortem interval, which included assaults, slips and falls and falls from heights, traffic accidents, and sharp instrumental injuries. Immunohistochemically, each kidney was stained against hemoglobin (Hb), myoglobin (Mb), superoxide dismutase (SOD), 8-hydroxy-2′-deoxyguanosine (8-OHdG), 150 kDa oxygen regulated protein (ORP150), pulmonary surfactant A (SP-A), and liver-type fatty acid binding protein (L-FABP). Bleeding or circulatory failure induced ORP150, 8-OHdG, and L-FABP in the kidney. Statistical analysis of the immunoreactivity revealed that in battered and/or abused cases, Hb could be considered a specific marker. Hb and Mb were observed in the cases with general severe trauma, such as slips and falls and falls from heights. In traffic accidents, ORP150 could reflect general circulatory failure with bleeding. SP-A was observed in the cases with severe thoracic injuries, such as lung injuries and multiple thoracic fractures. L-FABP appeared in cases with renal circulatory failure as well as renal injury. These findings suggest that immunohistochemical observation of the kidneys could be a useful tool in determining several key factors, such as the severity of injury, the specific damaged organ, and the pathological condition after injury.

1. Introduction

When investigating the cause of death in cases with multiple traumatic injuries, we consider the traumatic shock as a summation of multiple injuries. It is especially necessary to clarify the difference between the conditions of hemorrhagic shock, due to hemorrhaging from many wounds, and traumatic shock. Moreover, domestic abuse cases have increased in recent years. Thus, the clarification of the causal relationship between the injuries and cause of death in cases of abuse of adult women and the elderly is essential.

With the above factors in mind, it is important to diagnose the specific cause of death and mechanisms of trauma. It is also necessary to obtain useful information about the severity of the trauma, the main damaged organ or site, and the pathological condition after receiving the wound. Various investigations [1–4], especially immunohistochemical studies [5–10], about multiple traumas have been reported. Previously we reported that if Hb and Mb were observed in battered child cases, that this immunohistochemically reflected the additive trauma of repeated abuse [6]. We also reported that 8-OHdG and SOD, as a reaction to peroxidative damage, were induced in the kidneys by Mb in the case of rhabdomyolysis [11].

In the present study, we aimed to clarify the cause of multiple injuries and the post-traumatic pathophysiological condition or course. To this end, we observed the kidneys immunohistochemically with ORP150, L-FABP, SP-A and other biomarkers, in forensic autopsy cases.

2. Materials and methods

2.1. Cases

Sixty-three forensic autopsy cases were examined, all within 48 h of the postmortem interval. To demonstrate the difference between the conditions of hemorrhagic shock and traumatic shock, a control group (group A), consisting of 20 cases of death by bleeding from sharp instrumental injuries, was created. The multiple blunt injury cases were divided into three groups as

* Corresponding author. Address: Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. Tel.: +81 92 801 1011x3330; fax: +81 92 801 4266.
E-mail address: kuboshin@fukuoka-u.ac.jp (S.-i. Kubo).

1344-6223/$ - see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.legalmed.2011.09.003

Table 1
Summary of the examined cases.

Group		A		B		C		D		Total	
n	(clinical corse)	20	(8)	8	(2)	11	(10)	24	(4)	63	(24)
Sex	(M/F)	11/9		2/6		10/1		17/7		40/23	
Age	(Min–max)	50.2 ± 14.2	(27–71)	53.3 ± 19.0	(26–79)	48.9 ± 15.9	(27–69)	63.3 ± 19.6	(2–84)	55.4 ± 18.1	(2–84)
PMI	(Min–max)	19.5 ± 11.5	(7–48)	23.1 ± 13.4	(6–50)	32.5 ± 13.6	(14–48)	18.0 ± 7.5	(7–36)	21.6 ± 11.8	(6–50)
PTSI	(Min–max)	4.4 ± 9.8	(1–36)	7.7 ± 12.0	(1–36)	2.7 ± 2.5	(1–6)	9.3 ± 34.1	(1–168)	6.5 ± 22.0	(1–168)
ISS	(Min–max)	31.8 ± 18.0	(10–75)	28.3 ± 10.8	(11–42)	40.0 ± 21.6	(10–75)	53.8 ± 19.5	(16–75)	41.2 ± 21.0	(10–75)

The cases transported to the hospital are shown in ().
PMI: postmortem interval.
PTSI: post-traumatic survival interval.
ISS: injury severity score.

follows: Group B–battered and/or abused, eight cases; Group C–slips and falls or falls from heights, 11 cases; and Group D–traffic accidents, 24 cases. We observed cases involving falls separately from traffic accident cases in order to determine if they could be distinguished immunoreactively, even though both involve high energy wounds generated from multiple injuries. A summary of the examined cases is shown in Table 1. We estimated the severity of the trauma using the abbreviated injury scale (AIS) [12].

There were no significant differences in age, postmortem interval (PMI), and post-traumatic survival interval (PTSI) between the groups. However, the injury severity scores (ISS) in group D were significantly higher than group A and B ($p < 0.05$).

This study was executed in accordance with the privacy policy of the Japanese Society of Legal Medicine [13].

2.2. Immunohistochemical staining

The kidneys were fixed in phosphate-buffered formalin, embedded in paraffin and sectioned at 5 μm. Hematoxylin-eosin (HE) stain and Azan stain were used for conventional staining. Immunostaining was performed with antibodies against hemoglobin (Hb, 1:800, Dako, Japan), myoglobin (Mb, 1:800, Dako, Japan), 8-hydroxy-2′-deoxyguanosine (8-OH-dG, 1:200, JICA, Japan), superoxide dismutase Cu/Zn enzyme (SOD, pre-diluted, Lab Vision, USA), 150 kDa oxygen regulated protein (ORP150, 1:500, IBC, Japan), pulmonary surfactant type A (SP-A, 1:200, Dako, Japan), and liver-type fatty acid binding protein (L-FABP, 1:50, abcam, Japan). The immunostaining was carried out using the ENVISION kit/HRP (DAB) (Dako, Japan), according to the manufacturer's instructions. The immunostaining was visualized by incubation for 3–5 min with the DAB solution. For positive control tissue slices, muscle was used for Mb, liver was used for 8-OHdG and SOD, lung was used for SP-A, and kidney was used for L-FABP. Staining specificity was checked using negative control slides omitting the primary antibody. Additionally, tissue specimens other than positive control tissues were used in a negative control study. The coverslip was mounted on a glass slide, examined and photographed. Controls for the specificity of the immunohistochemistry involved omission of the primary antibody.

2.3. Immunohistochemical scoring

The immunoreactivity was screened semi-quantitatively by observing the pattern and the number of positive cells in all fields of a whole section. Immunostaining patterns were divided into two grades as follows: (1) the diffuse staining type, where the positive cells were spread out across the section (D-type, 2 points); and (2) the focal staining type, where the positive cells were clustered (F-type, 1 point). They were then evaluated according to the number of immunopositive cells, and classified into two grades as follows: (1) a large number of immunopositive cells (L-type, 2 points); and (2) a small number of immunopositive cells (S-type, 1 point). No immunoreactivity was assessed as negative, and given zero points. A final value was assigned by multiplying the points from the immunostaining pattern with the points from the number of immunopositive cells, and this value was adopted as the immunoreactive score [14].

The pathological findings of the kidneys were observed separately by two pathologists, and were then screened.

2.4. Statistical analysis

The Mann–Whitney *U* test was utilized to compare the immunoreactive scores against the decedent's sex. A *p*-value of less than 0.01 was considered statistically significant. The correlation coefficients were analyzed by Spearman's rank coefficient of correlation between the immunoreactive score and each of the following: the injury severity score, the decedent's age, the postmortem interval, and the post-traumatic survival interval. The immunoreactivity scores of each group were analyzed with the Mann–Whitney *U* test.

3. Results

The immunoreactivities against each antigen are summarized in Table 2. In addition to the data of Table 2, in the 21 SP-A positive cases, lung injuries were present in 12 of them (57.1%). Seventeen cases had an AIS score of four or more (81.1%). In the eight liver injury cases, L-FABP was positive in 75% of them (six cases). However, L-FABP was observed in all six of the kidney injury cases (100%).

The localization of each Hb, Mb, ORP150, and L-FABP was observed in the cytoplasm of tubular cells, and the lumen of the tubules and the glomerulus. 8-OHdG was observed in the nucleus of both tubular and glomerulus cells. The localization of SOD was observed in the cytoplasm and nucleus of tubular cells, and the lumen of the tubule. The localization of each SP-A, was observed only in the lumen of tubulus.

The immunoreactive patterns for the antigens Hb (Fig. 1a), SOD (Fig. 1d), and SP-A (Fig. 1f) displayed the F-type pattern about 60% or more of the time. For both ORP150 (Fig. 1e) and L-FABP (Fig. 1g), the D-type pattern was observed about 60% or more of the time. In Mb (Fig. 1b) and 8-OHdG (Fig. 1c), there was no great difference between the frequency of D-type and F-type patterns. Regarding the number of immunopositive cells, most of the antigens were of the S-type, except for Mb and 8-OHdG, in which the S-type and L-type frequencies were almost the same.

No correlation was found between the immunoreactivity scores for these antibodies and the cadaver's age, injury severity score, the postmortem interval, or post-traumatic survival interval. There