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Determination of Survival Time Following Isolated Head Trauma by Histopathological Method

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Objectives: The objectives of this study were determine the demographic characteristics of patients who died from isolated head injuries having no surgery and to determine their survival time based on histopathological changes in the injury hematoma and brain. **Methods:** Between 2016 and 2017 the Department of Forensic Medicine of the National Institute Forensic Science recorded 3640 deaths of which 757 cases had skull and brain trauma. A subset of 372 of these patients with isolated head trauma who did not have surgery were analyzed. The tissues from 30 of these patients underwent histopathological analysis to determine the time from injury to death. **Results:** Out of 372 cases studied, 152 (83%) were male, 32 (17%), were female (male to female ratio 5:1) and most (n=53, 28.8%) were within 40-49 years of age (mean age 42±15.9). Trauma to the anterior head was the most common location of injury (257, 69.1%). We identified 30 cases to estimate time from injury to death by histological examination using Martius Scarlet Blue stain. Among these cases 10 died between 0-6 hours after head trauma, 2 died between 6-12 hours, 13 cases died between 12-18 hours, 3 cases died between 18-24 hours, 1 case died 24-48 hours, and 3 cases died 48 hours after head injury. **Conclusion:** Males who suffered death from traumatic head injuries from blunt objects were most frequently identified in our research. Brain edema, subarachnoid hemorrhage, frontal skull fracture and brain contusion were most common. The interval of time from the head trauma to death was determined by the age of the fibrin using histochemistry stain.

Keywords: Skull and Brain Trauma, Survival Time, Forensic Medicine, Histopathology, MSB (Martius Scarlet Blue).

Introduction

Head injury can cause morphologic changes of the brain's anatomical structures[1]. Types of injury caused by different mechanisms of injury are skull fracture, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cortical hemorrhage, intracerebral hemorrhage, intraventricular hemorrhage, gliding contusion injury, diffuse axonal injury, and concussion injury[2].

Neurotrauma is a critical public health problem that deserves the attention of the world's health community. Estimates of the occurrence of brain and spinal cord injury indicate that these injuries result in enormous losses to individuals, families, and communities. They result in a large number of deaths and impairments leading to permanent disabilities. More than 50% of all death cases encountered during forensic autopsy are associated with brain trauma[3].

One of the most common complications of head trauma is hemorrhage. Hemorrhage is a result of bleeding from the veins between the dura mater and the arachnoid of the brain which occurs in about 30% of cases. Arterial bleeding is more rapid, thus resulting in the quick onset of clinical symptoms and fatal outcomes[3].

For the past 10 years, accidents and trauma rank third in the cause of death in the Mongolian population[4]. The National Institute of Forensic Science reported registered 4016 deaths in the Forensic Medicine Department in 2017 and or these 235 were blunt trauma, 625 were traffic accidents, 29 were trauma by firearm, 80 other penetrating trauma, 116 were fall from height, 534 were asphyxiation, and 807 were intoxication[5].

According to the data from The State Forensic Medicine Service, in Lithuania, injuries with blunt objects are the 3rd (16%) most common cause of death from the unnatural causes, with a male-to-female ratio of 2.44:1. Asphyxiation and intoxication were the most common causes of death. Russian scientists have determined that skull and brain trauma comprise of 40% of total trauma and that skull and brain trauma are one of the serious injuries that can have damaging effect to health[6].

Head injuries are the main cause of death and account for one-third to one-half of traumatic deaths[7]. Head injuries may be divided into primary and secondary. Primary injury relates directly to the trauma; secondary injury refers to the secondary development of brain edema, compression of the brain, secondary hemorrhage, especially in the brain stem, and brain herniation[8].

Implementing contemporary methods to determine the mechanism of injury, how long a person survived afterwards, and the cause of death are urgently needed in the science of forensic medicine in Mongolia. Analysis of the origin and patterns of traumatic head injury using state of the art forensic medicine is critical to determine the time of the injury and duration of life afterwards when there are no witnesses describing what happened and there is minimal evidence from other investigations.

Foreign researchers have determined the temporal and spatial sequence of events that follow head injury, their impact on the central nervous system including the decline of blood flow in general and specific areas, and the pathology of blood vessel wall based on the origin of the injury[4]. Namely, injured tissues expose tissue factor which activates the blood clotting system that attract platelets to the injury site and convert soluble fibrinogen in the serum into fibrin. Fibrin forms long stands of tough insoluble protein that then cross link, contract, and harden forming a three-dimensional mesh in a finely tuned sequence of reactions that can aid the forensic scientist in determining the elapsed time from injury to death using histopathological methods[9].

In other countries, the application of forensic science on intracranial hemorrhage is of special interest in everyday practice[10]. But in Mongolia the forensic medical experience on intracranial hemorrhage has been limited and we know of no usage of histopathological methods to determine the period of time between head injury and death by tissue analysis.

The objectives of this study were determine the demographic characteristics of patients who died from isolated head injuries having no surgery and to determine their survival time using a histopathological method.

Materials and Methods

Study design and sampling

In 2016 and 2017 the Department of Forensic Medicine of the National Institute Forensic Science recorded 3640 deaths of which 757 cases had brain trauma. Of the 757 cases, 372 cases died with only brain trauma. Because 84 of these cases patients had surgery at the National Trauma and Orthopedic Research

Center and other hospitals in Ulaanbaatar, they were excluded from the study. Therefore, our inclusion criteria were cases of only skull and brain trauma who did not have surgery. Thirty cases were with subdural hematoma and brain tissue in paraffin embedding molds were identified. We conducted a retrospective study of these 30 cases to determine the time interval from injury to death using hematoxylin and eosin main staining, and Martius Scarlet and Blue (MSB) histochemical staining of the trauma hematoma and adjacent tissues.

Statistical analysis

Demographic data were summarized as mean, and standard deviation. The association between percentile variables were analyzed using Chi-Squared test, and the Fisher exact test. For purposes of analysis, the subjects were grouped into the following ages: below 29 ages, 30-39 ages, 40-49 ages, over 50 ages. Differences with p-values < 0.05 were considered significant. All analyses were performed using Statistical Package for Social Sciences (SPSS 25.0) software.

Histological methods

To evaluate the time of injury, histologic examination of subdural and epidural hematomas and adjacent tissues were performed. Sections were cut and prepared for routine light microscopy. The histomorphologic features of the dura and the clot were examined using hematoxylin and eosin stain. MSB stain was used for the detection of collagen fibers.

The hematoxylin and eosin stain consisted of several procedures. The sections were dehydrated with absolute alcohol within 7-minutes, following hydration with 90%, 70% ethanol, and distilled water for 7-minutes. The hematoxylin stain was applied for 6-minutes, followed a continuous wash in running water for 5-minutes, and cleaning with 96% ethanol for 1-minute. Second, eosin and floxin solution was applied for 5-minutes. Finally, a quick dehydration with 70%, 90%, and absolute alcohol was performed for 6-minutes. Using this protocol, the nucleus stained a blue-violet color; cytoplasm and matrix were stained in different pink tones.

MSB Staining: Deparaffinized sections were obtained using xylene an ethanol, and then rehydrated with water. If the specimen had been previously fixed with mercury, the mercury was removed with the iodine-thiosulphate sequence. If the specimen had not been fixed with mercury, it was placed into Bouin's fluid at 56°C for 1 hour and then rinsed well in distilled water. The nuclei were stained with an acid resistant nuclear stain after which the specimen was rinsed with 95% ethanol. It was then placed in martius yellow stain for 2 minutes and then rinsed with distilled water. After this, it was placed in crystal scarlet stain for 10 minutes. It was then differentiated with phosphotungstic acid until only fibrin was red (up to 10 minutes). It was then placed in methyl blue until the collagen was blue (up to 10 minutes) after which it was briefly rinsed with 1% aqueous acetic acid. The specimen was then dehydrated rapidly with ethanol and cleared with xylene and mounted with a resinous medium. Using this protocol, the fibrin stains different colors visible by light microcopy depending on its age as follows: 0-6 hours fresh - yellow, 6-12 hours – orange, 12-18 hours - red, 18-24 hours purple, 24-48 hours gray, and more 48 hours old - blue. The erythrocytes stain yellow, and the connective tissue blue[6].

Ethical statements

Bioethical permission for this research was given by the Biomedical Ethics Committee of Mongolian National University of Medical Sciences, May 22, 2015 (Number of ethics permission: 15/3/201515).

Results

Cause of death and risk factors associated with mortality from skull and brain trauma

Of the 372 cases studied, 83.9% (n=312) were male and 16.1% (n=60) were female, with a gender ratio 5:1. In our study, 92.7% (n=345) of tissues studied were taken from patients with closed head injuries, and 7.3% (n=27) were open head injuries. Most of injuries in our study occurred in 2017 (58.1%, n=216) although there were a substantial number from 2016 (41.9%, n=156).

Regarding the age at the time of death, 13.2% (n=49) were between 20-29 years of age, 22% (n=82) were between 30-39, 26.1% (n=97) were between 40-49 and 21.8% (n=81) were between 50-59 years of age. The mean age was 41.5±15.9 (Figure 1).

Of the 286 males, 91.9% were between 20-69 years of age while of the 47 females who died 78.8% were 20-59 years of age. No statistically significant difference in the age groups was found between genders (p=.067).

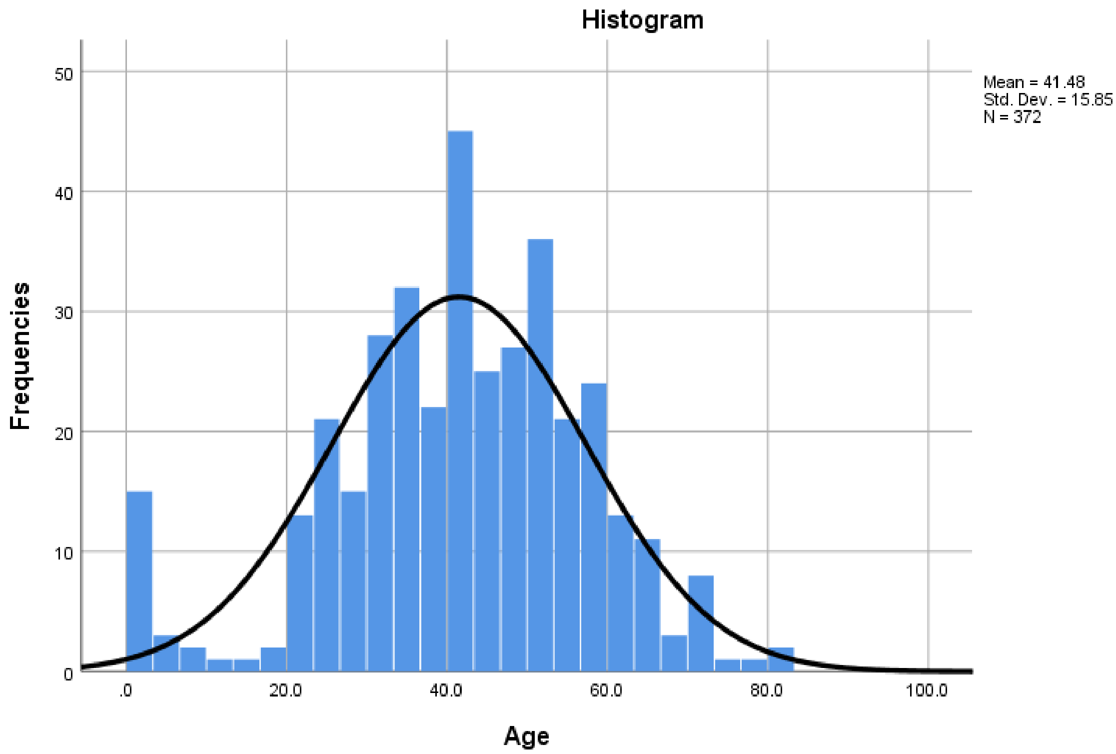


Figure 1. Ages of the 372 patients with isolated head trauma who did not have surgery.

Table 1. Association of gender and hospitalization status at time of death of patients with isolated head trauma who did not have surgery.

Status n	Total Female		Male	Gender			p-value
	%	n	%	n	%		
							.005[P]
Hospitalized	180	48.4%	19	31.7%	161	51.6%	
Not hospitalized	192	51.6%	41	68.3%	151	48.4%	
Total	372	100.0%	60	100.0%	312	100.0%	

P - Pearson Chi-Square test

Table 2. Association of causes and gender of patients with isolated head trauma who did not have surgery.

Cause	Total	Female		Male	Gender		p-value
	n	%	N	%	n	%	
							.233[f]
Traffic accident	79	21.2%	15	25.0%	64	20.5%	
Blunt trauma	257	69.1%	38	63.3%	219	70.2%	
Other	36	9.6%	7	11.7%	29	9.3%	
Total	372	100.0%	60	100.0%	312	100.0%	

f- Fischer Exact Test

Table 3. Association of causes and age group of patients with isolated head trauma who did not have surgery.

Cause	Blunt force trauma		Traffic accident value		Other		p-value
Age	n	%	n	%	n	%	
							0.0001[f]
Below 29	30	11.7%	29	36.7%	14	38.8%	
30-39	54	21%	19	24%	9	25%	
40-49	76	29.6%	13	16.5%	8	22.2%	
Over 50	97	37.7%	18	22.8%	5	14%	
Total	257	100%	79	100%	36	100%	

f- Fisher Exact Test

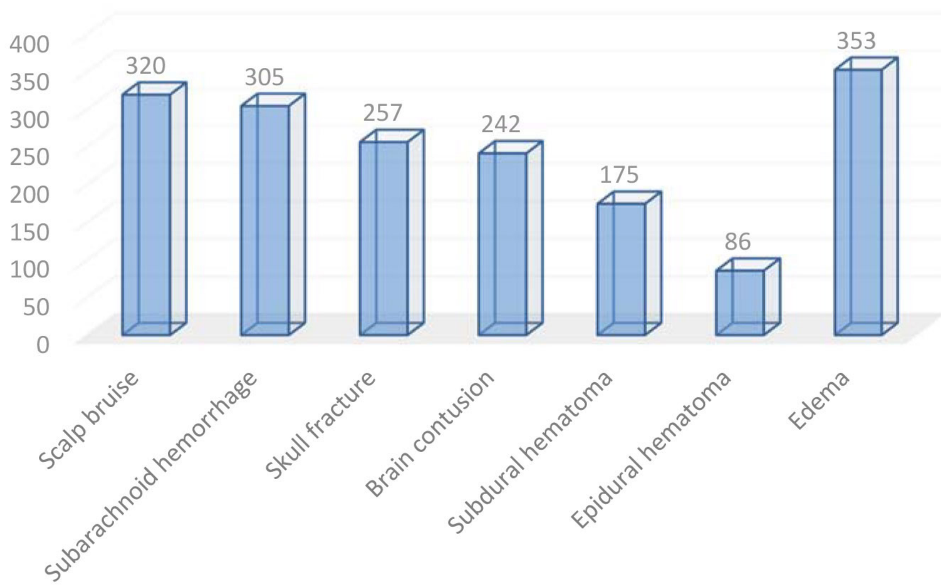


Figure 2. Pathological findings of skull and brain trauma of patients with isolated head trauma who did not have surgery.

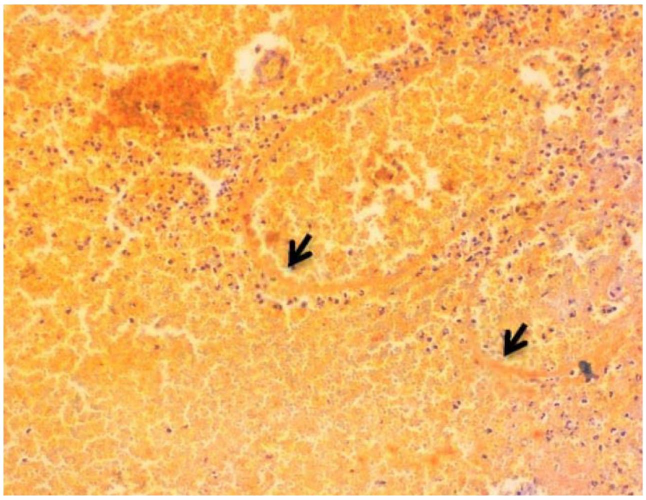
In 51.6% (n=192) of cases, victims of head trauma died prior receiving any medical treatment, however 48.4% (n=180) died at the hospital (Table 1). Women died more frequently prior to hospitalization than men (68.3% vs. 48.4%, p=0.005).

Most of the specimens (69.1%) were from people with blunt head trauma while 21.2% had head trauma as a result of traffic accident. Other mechanisms of injury included falls from a height (8.6%), train wrecks (0.5%), while penetrating trauma was rare (0.5%). No statistically significant difference was found between cause of trauma and gender (p=0.233) (Table 2). But, there was a statistically significant difference comparing age groups with cause of trauma (p=0.0001). Most of the causes of trauma in people 20-29 years of age were falls from height, ages 30-39 were traffic accidents, and for people 40-49 years of age was blunt trauma other than traffic accidents (Table 2).

The characteristics of the head and brain injury were tabulated and 95% were found to have cerebral edema, 82% had subarachnoid hemorrhage, 69% had skull fracture, 65% had brain contusion, 47% had subdural hematoma, 23% had epidural hematoma, and 6% had scalp bruises (Figure 2). No statistically significant difference was found in the characteristics of pathology and cause of trauma, or the group of ages and gender (p>.05).

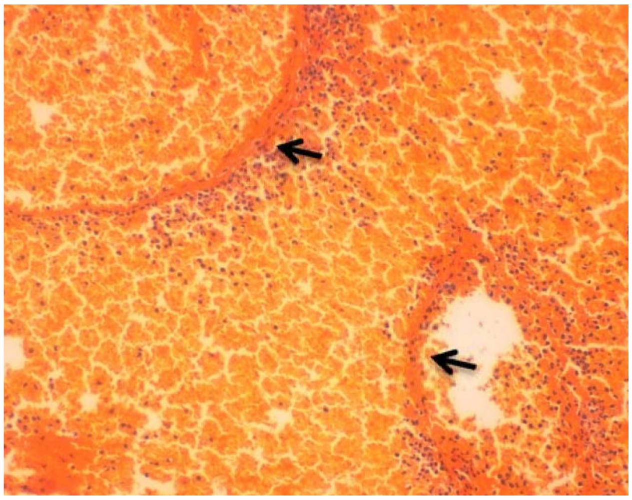
Determination of survival time of skull and brain trauma by histopathological method

Hemolysis, blood congestion in vessels, vessel thrombus,



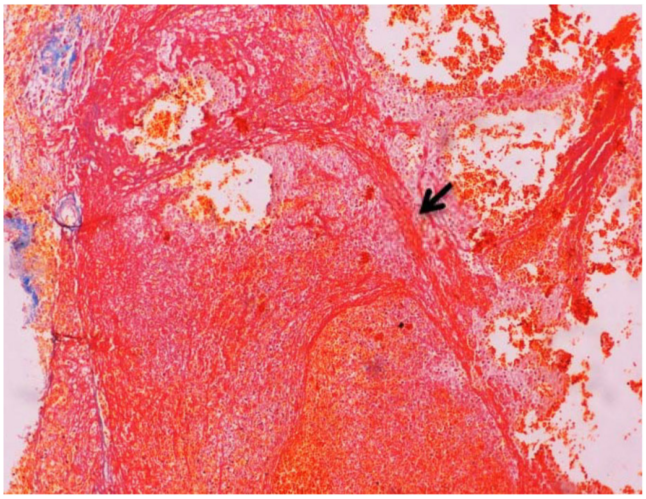
MSB stain. 100x (100-power magnification)

Figure 3. 0-6hours yellow fibrin (arrows)



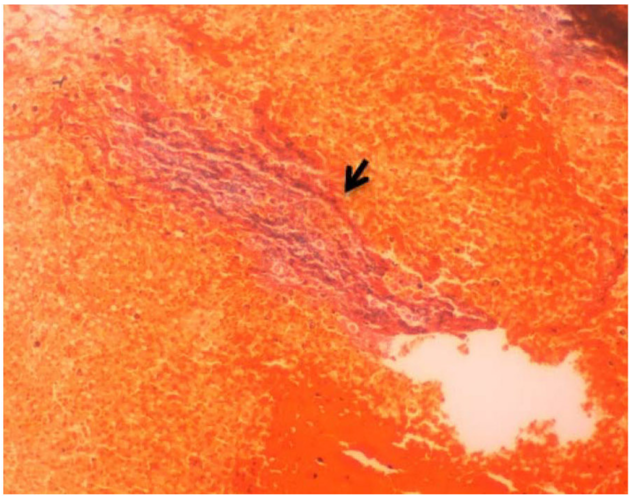
MSB stain. 100x

Figure 4. 6-12hours orange fibrin (arrows)



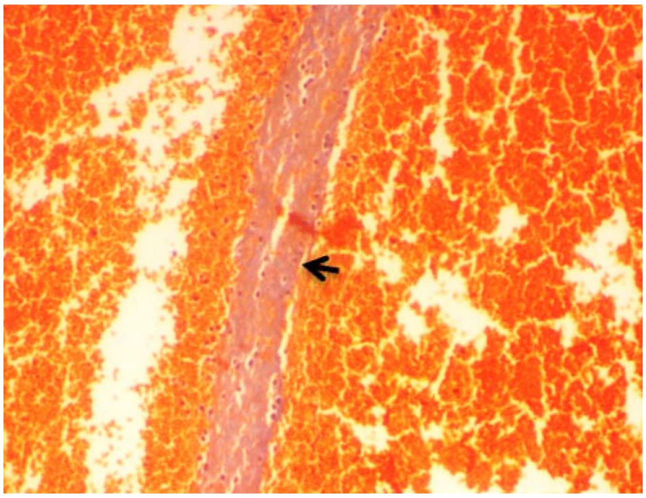
MSB stain. 100x

Figure 5. 12-18hours red fibrin (arrow)



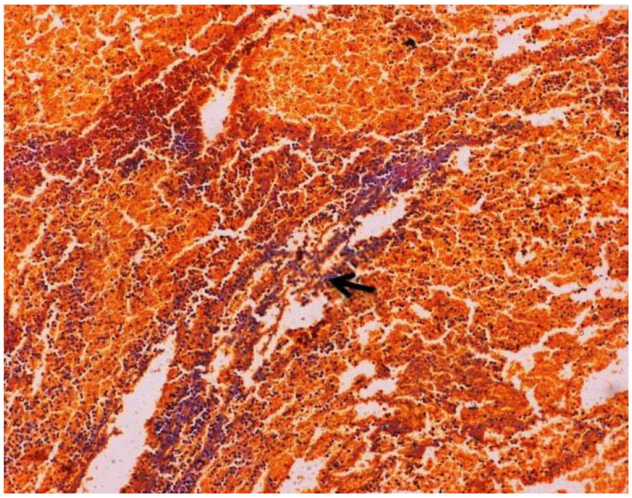
MSB stain. 100x

Figure 6. 18-24hours purple fibrin (arrow)



MSB stain. 200x

Figure 7. 24-48hours gray fibrin (arrow)



MSB stain. 100x

Figure 8. >48hours blue fibrin (arrow)

infiltration of leucocyte, fibrin, infiltration of macrophage and growth of connecting tissues at the origin of brain trauma were identified with the hematoxylin and eosin stain 0-6 hours, 6-24 hours, 24-72 hours, more than 4 days of injury. 59% of them passed away 6-24 hours of injury.

We identified 30 cases in which to estimate time interval between head injury and death by histochemical method. This was done using the MBS histological staining examination described previously. There were 8 cases in which the fibrin stained yellow (Figure 3) indicating that death occurred within 0-6 hours, 2 cases in which the fibrin stained orange (Figure 4) indicating that death within 6-12 hours of injury, 13 cases in which the fibrin was red (Figure 5) indicating 12-18 hours and, 3 cases with purple fibrin (Figure 6) indicating 18-24 hours, 1 case with gray fibrin (Figure 7) indicating 24-48 hours, and 3 cases in which the fibrin stained blue (Figure 8) indicating that death occurred more than 48 hours after the head injury.

By the method of histochemical MSB stain bright red fibrin was observed most frequently indicating that 43.3 % of them died within 12-18 hours after getting skull and brain trauma.

No statistically significant difference was found survival time and gender (p=0.446), survival time and age group (p=0.531), or survival time and cause of trauma (p=0.417).

Discussion

Damage of the brain due to head trauma is differentiated according to whether the injury is open or closed, i.e. the coverings of the brain are intact or penetrated and the result is called open or closed head injury respectively. A closed brain injury is characterized by an intact skull and dura mater; in contrast, an open brain injury usually is characterized by a laceration of skin, fracture of the skull and laceration of dura mater[11]. Closed head injuries were identified in 92.7% (n=345) of the patients in our study. This was the result of the high frequency of blunt trauma without traffic accident and traffic accidents identified as the mechanisms of injury identified in our study.

Fresh coagulated blood between the arachnoid mater and the dura mater is a characteristic feature of acute subdural hematoma and can be diagnosed by clinicians or pathologists during the first 3 days following the traumatic event[2]. The different localization of the hemosiderin-containing macrophages in the brain mater layers is an indicator of several bleeding episodes[2]. The subacute phase of subdural hematoma begins 3 to 7 days after acute injury, but it often takes than 3 weeks for the appearance of clinical symptoms[2,12-15].

Coagulation of blood in the area of a brain injury begins with activation of the coagulation cascade and the recruitment of platelets. As part of the coagulation process, fibrin forms a three-dimensional mesh which holds a hematoma's components together[2.] This is followed by the migration of leukocytes, ingestion of erythrocytes (phagocytosis) as demonstrated by iron-containing macrophages. Later there is formation of granulation tissue, collagenous fibers, endothelial cells and remodeling of the tissue by macrophages[2]. The steps that occur in a known and predictable sequence and the times at which they occur are known and can be studied using histological methods.

Using histopathological stains, the elastic fibers and elastic laminae were stained purple/grey and they could be easily distinguished from collagen fibers which stained blue. The addition of Orcein to the MSB staining allowed clear identification of even the finest elastic fibers and the staining was not masked or abolished by other dye solutions. Erythrocytes located in the lumen of the vessel and those embedded in plaques were stained yellow[16]. Therefore, based on these, it was possible to define the survival time depending on changes in the hematoma due to skull and brain trauma by MSB stain. MSB staining method discriminated fibrin and selected the other components in the hematoma.

In the study of traumatic brain injuries by Tallon et al. in the USA, the most common abnormalities identified were subarachnoid hemorrhage (64%), intra parenchymal hemorrhage (57%), and subdural hemorrhage (40%)[17]. However, in our investigation of fatal head injuries subarachnoid hemorrhage was more common at 80.4%, followed by intra parenchymal hemorrhage at 20.7% and subdural hemorrhage at 45.7%.

The study by Nedugov et al. determined the age of subdural hematoma using histopathological methods in 2011[18]. Their age criteria was based on the formation the capsule of the hematoma, new capillaries in the capsule, macrophage infiltration, lymphocyte and plasmocytes in the capsule and hemosiderosis of capsule.

In the study by Chmieliauskas et al. the most common mechanism of injury of head trauma was fall of an upright person to the ground (60.9%)[3,19]. Other less frequent causes were a blow to the head with blunt object (12.7%) and a fall from a height (10.9%). In 57.4% of cases, skull fractures were identified. In 83.6% of cases, focal cerebral contusions were visible. In 63.6% of cases, bleeding under the pia mater was identified. Approximately 92% of patients that were hospitalized had symptoms of brain edema. Blood under the dura mater was identified in 80% of cases. In 14.3% of deaths, patients did not receive any medical help prior to death which occurred within 1 to 3 hours after the injury. In 8.9% of cases, death occurred between 3 and 6 hours after injury and in 30.4% of cases death occurred within 6 to 12 hours. These results were established based on the autopsy results and histochemical result by Masson trichrome stain. In the patients in our study, we determined that 59% passed away 6-24 hours later after injury by hematoxylin eosin stain, however 43.3 % of them died within 12-18 hours by MSB stain.

Our study has certain limitations. First, the study subjects were derived from Ulaanbaatar's population and our results are not generalizable to other locations within Mongolia or elsewhere. Second, we did not study surgical biopsy specimens, because death after surgery could be attributed to the surgical or postoperative causes. Third, we did not attempt to compare the reported interval of time between injury and death with the results we obtained using histopathological stains because the former was often a reason for the forensic investigation.

Looking forward, it is important for us to assess the morphologic and histological features of brain tissues after death when patients are not hospitalized and do not receive neurosurgeon's examination. We will continue our research in this field and will precisely investigate the histological features of brain tissue by immunohistochemical stain.

Further research is needed using more detailed methods to determine survival time of head injury, for example, using other histochemical stains and immunohistochemical stains on traumatic hematoma. The association of the microscopic morphology of changes to the brain that occur with time following head injury are still an area of scientific debate in forensic medicine.

Conclusion

Males who suffered death from traumatic head injuries from blunt objects were most frequently identified in our research. Brain edema, subarachnoid hemorrhage, frontal skull fracture and frontal brain injury were most common. The interval of time from the head trauma to death was determined by the age of the fibrin using contemporary histochemistry staining methods in Mongolia, which to our knowledge have not been done in our country previously.

Conflict of Interest

The authors state no conflict of interest.

Acknowledgements

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