

## ORIGINAL RESEARCH

## The Effect of Blood Loss in the Presence and Absence of Severe Soft Tissue Injury on Hemodynamic and Metabolic Parameters; an Experimental study

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### Abstract

**Introduction:** The effect of severe soft tissue injury on the severity of hemorrhagic shock is still unknown. Therefore, the present study was aimed to determine hemodynamic and metabolic changes in traumatic/hemorrhagic shock in an animal model. **Methods:** Forty male rats were randomly divided into 4 equal groups including sham, hemorrhagic shock, soft tissue injury, and hemorrhagic shock + soft tissue injury groups. The changes in blood pressure, central venous pressure (CVP) level, acidity (pH), and base excess were dynamically monitored and compared. **Results:** Mean arterial blood pressure decreased significantly in hemorrhagic shock (df: 12; F=10.9; p<0.001) and severe soft tissue injury + hemorrhagic shock (df: 12; F=11.7; p<0.001) groups 15 minutes and 5 minutes after injury, respectively. A similar trend was observed in CVP in severe soft tissue injury + hemorrhagic shock group (df: 12; F=8.9; p<0.001). After 40 minutes, pH was significantly lower in hemorrhagic shock (df: 12; F=6.8; p=0.009) and severe soft tissue injury + hemorrhagic shock (df: 12; F=7.9; p=0.003) groups. Base excess changes during follow ups have a similar trend. (df: 12; F=11.3; p<0.001). **Conclusion:** The results of this study have shown that the effect of hemorrhage on the decrease of mean arterial blood pressure, CVP, pH, and base excess is the same in the presence or absence of soft tissue injury.

**Key words:** Shock, hemorrhagic; soft tissue injuries; hemodynamics

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### Introduction:

Hemorrhage is responsible for about 40% of trauma deaths, 33% to 56% of which happen during the pre-hospital period (1). On average, 1 in every 4 patients affected by severe trauma, suffers from coagulopathy. Coagulopathy and severe hemorrhage plays an important role in determining the final outcome of the patient, their mortality and disability (2-4). Soft tissue injury is one of the most important injuries that can lead to coagulopathy (5-9) as it can activate both the coagulation pathway and the fibrinolytic system (10). But the effect of severe tissue injury on the severity of hemorrhagic shock is still not known. Only in one study it has been demonstrated that the existence of severe tissue injury can decrease the cardiovascular responses in shock and correct bradycardia and hypotension to some extent (11). But other studies have ex-

pressed that the physiologic responses observed in traumatic hemorrhagic shock are no different from other kinds of hemorrhagic shock (12-14).

To realize the pathophysiology of traumatic/hemorrhagic shock, reductionistic approaches such as animal models were used to study the physiology of "normal" states, to compare with pathologic conditions to follow the alterations after modulation of pathologic states (15-17). A traumatic/hemorrhagic shock animal model was used to simulate the pathophysiological changes characterizing the disease entity in trauma patients with hypovolemic shock (18-22). Therefore, this study was carried out to determine hemodynamic and metabolic changes in traumatic/hemorrhagic shock rat model which can be a clinical testing to clarify the process in human patients.

### Methods:

#### *Study design and setting*



The present experimental study was conducted in accordance with the guidelines of ethics for the evaluation of pain in conscious animals. Animal selection, experiments, subsequent care and the sacrifice method were all in line with the guidelines of Animal Care Committee of Iran Veterinary Organization. All experiments were performed under aseptic conditions in Laboratory Animal Center of Shiraz University of Medical Sciences. The study was approved by the ethics committee of the institution. The duration of trials and the number of animals used were reduced to a least possible to minimize animal pain and suffering. During the experiments, the animals were kept one per cage, maintained under controlled environmental conditions ( $21 \pm 2^\circ\text{C}$ , 65–70% relative humidity; 12 hour light/dark cycle started at 7:00 Am) and had free access to water and food. Before the experiments, all the animals were deprived of food for 4 hours, but had free access to water to prevent excessive dehydration during starvation. At the end of the procedure, the rats were euthanized under anesthesia or carbon dioxide.

#### Animals

40 male Sprague-Dawley experimental rat models (weight, 200-250 gram) were randomly divided into 4 equal groups: sham (catheter insertions were done, without shock induction), hemorrhagic shock, severe soft tissue injury, and severe soft tissue injury + hemorrhagic shock. Animals that died during surgical procedures and rats that lost more than 0.2 mL blood during catheterization or did not have spontaneous breathing 10 minutes after the procedure were excluded.

#### Interventions

In all procedures, rats were anesthetized with 60 mg/kg intra-peritoneal pentobarbital (Sigma chemical company, St, Louis, USA) with a 10 mg/kg as maintenance dose (23). Body temperature during anesthesia, catheterization, hemorrhage shock, and soft tissue injury was controlled by a heat pad and preserved at  $37.0^\circ\text{C}$ .

##### A) Catheterization

After the confirmation of deep sedation (no response to noxious stimulus) and stability of respiratory rate, three catheters were inserted into femoral artery, carotid artery, and jugular vein (tip of this catheter were placed in the right atrium) for assessing the arterial blood gas sample, blood pressure, and central venous pressure, respectively. In addition, the hemorrhagic shock was induced from the femoral catheter.

Lidocaine (5mg/kg subcutaneously) was locally used before placing the catheters. Catheterization was done with polyethylene tubes number 50 (PE50) cannula (Becton Dickinson, Sparks, MD; United States). Carotid artery, and jugular vein catheters were connected to pressure transducers and blood pressure analyzer (Micro-Med, Louisville, KY; United States) for continuous monitoring of animals' hemodynamic state (24). All the catheters

were fixed in place using surgical silk sutures. After catheterization, all animals were allowed to stabilize (30 minutes) and baseline hemodynamic parameters were assessed and blood samples were taken (0.2 mL, by insulin syringes).

##### B) Inducing the hemorrhagic shock

After the stabilization of animals and baseline assessment, hemorrhagic shock was induced using a fixed-pressure model. For this purpose, the researcher removed 1 mL blood every minute through the femoral artery catheter, using a 2-mL syringe (Pars syringe, Tehran, Iran). The blood removing was done five times with 10 minute intervals for tuning blood pressure. Bleeding was continued until the mean arterial blood pressure reached 25-30 mmHg. Heparin (500 U/kg) was intravenously injected through tail vein to prevent blood clotting (24).

##### C) Soft tissue injury

A close femoral bone fracture and soft tissue injury with no hemorrhage (contusion model) were induced using a blunt guillotine ramming system with a dropped steel weight (0.5kilogram; from a drop height of 15cm). To ensure postoperative stabilization, intramedullary stabilization was performed using a steel pin (24).

#### Outcome

During the experiment, the changes in blood pressure (BP), central vein pressure (CVP), blood acidity (pH), and base excess (BE) were dynamically monitored during 60 minutes.

#### Statistical analysis

Data were analyzed using SPSS software (Version 13, Chicago, IL, USA) and were expressed as mean  $\pm$  SEM. Within-group comparisons were performed by repeated-measures analysis of variance (ANOVA) and inter-group comparisons were done using two-way ANOVA and Tukey post hoc test. P value of less than 0.05 was considered statistically significant.

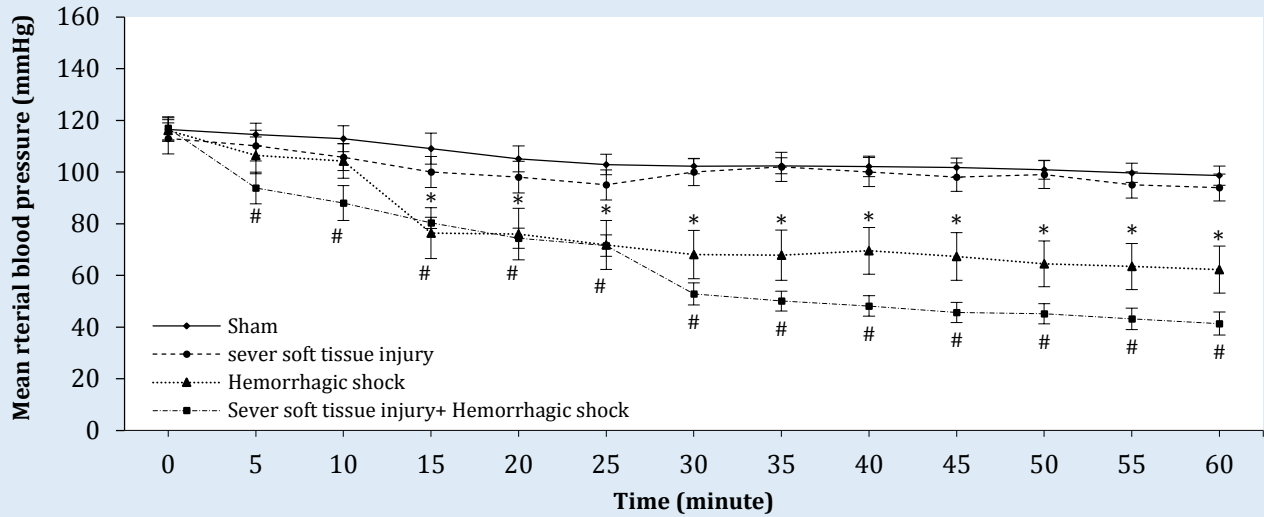
#### Results:

Mean arterial blood pressure significantly dropped in the hemorrhagic shock (df: 12;  $F=10.9$ ;  $p<0.001$ ) and severe soft tissue injury + hemorrhagic shock (df: 12;  $F=11.7$ ;  $p<0.001$ ) groups 15 minutes and 5 minutes after injury, respectively, and it continued until 60 minutes. This decrease was significant compared to the sham ( $p<0.001$ ) and soft tissue injury ( $p<0.001$ ) groups (Figure 1).

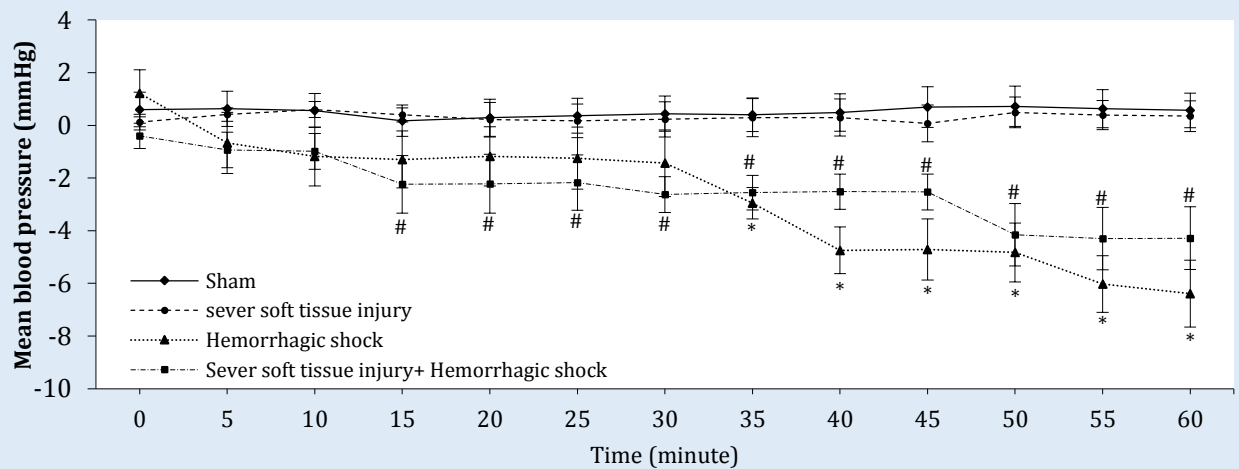
A similar trend was observed in CVP in the severe soft tissue injury + hemorrhagic shock group (df: 12;  $F=8.9$ ;  $p<0.001$ ) and in the hemorrhagic shock (df: 12;  $F=6.4$ ;  $p<0.001$ ). CVP was significantly reduced from 35 minutes after injury (df: 12;  $F=7.6$ ;  $p=0.005$ ). This decrease was significant compared to the sham ( $p<0.001$ ) and soft tissue injury ( $p<0.001$ ) groups (Figure 2).

After 40 minutes, pH was significantly lower in the hemorrhagic shock (df: 12;  $F=6.8$ ;  $p=0.009$ ) and severe soft





**Figure 1:** Comparison of mean arterial blood pressure between the experimented groups. \*, shows significant difference of hemorrhagic shock group with the sham group at the same time. #, shows significant difference of severe soft tissue injury + hemorrhagic shock group with the sham group at the same time.



**Figure 2:** Comparison of central venous pressure between the experimented groups. \*, shows significant difference of hemorrhagic shock group with the sham group at the same time. #, shows significant difference of severe soft tissue injury + hemorrhagic shock group with the sham group at the same time.

tissue injury + hemorrhagic shock (df: 12;  $F=7.9$ ;  $p=0.003$ ) groups compared to the sham group (Figure 3). Base excess changes during follow ups show a similar trend. Compared to the sham animals, hemorrhagic shock (df: 12;  $F=11.3$ ;  $p < 0.001$ ) and severe soft tissue injury + hemorrhagic shock (df: 12;  $F=10.1$ ;  $p < 0.001$ ) induction lead to significant decrease in base excess in 40 minutes and 60 minutes after injury (Figure 4).

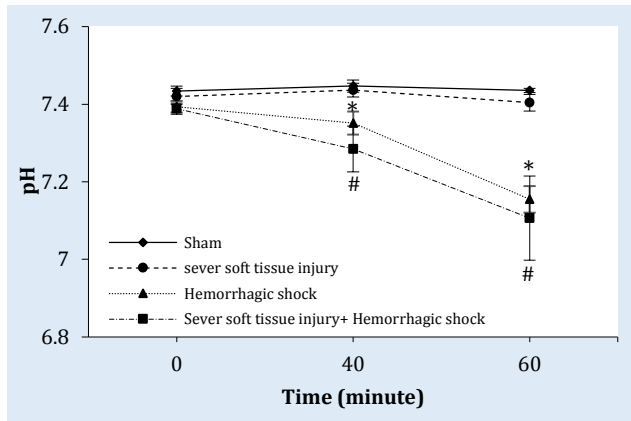
#### Discussion:

The results of this study have shown that the effect of hemorrhage on the decrease of mean arterial blood pressure, central vein pressure, blood acidity, and base excess is the same in the presence or absence of soft tissue

injury. In this regard, we can say that soft tissue injury in the absence of hemorrhagic shock has no effect on hemodynamic and metabolic parameters of the blood.

One of the major causes of mortality in traumatic patients, is hemorrhagic shock. Therefore, evaluating the factors affecting the severity of the shock is of great importance. In the current study, the presence of severe soft tissue injury was studied as a factor that potentially affects hemorrhagic shock. The results from this study showed that severe tissue injury has no effect on blood pressure, central vein pressure, blood acidity, and base excess. While Little et al. express that the presence of severe soft tissue injury can influence the cardiovascular





**Figure 3:** Comparison of blood pH between the experimented groups. \*, shows significant difference of hemorrhagic shock group with the sham group at the same time. #, shows significant difference of severe soft tissue injury + hemorrhagic shock group with the sham group at the same time.

response to hemorrhagic shock (11). The reason for this difference might be that Little et al. had used ischemia to induce the injuries while most soft tissue injuries induced by crush injury. The mechanisms involved in these models are different and probably that is the reason for the difference seen in the cardiovascular response between the present study and the Little et al. study (24). Our model of blood withdrawal in the current study combined with active bleeding could produce a severe and reproducible hemorrhagic shock. To date, many hemorrhagic shock-resuscitation models have been established, but none of them covered the important aspects regarding severity, clinical reality, and reproducibility (25-27). Our method described here and our experimental findings have led to the creation of a severe but reliable and realistic acute hemorrhagic shock model in rats. In the present experiment, rats reliably survived a hemorrhagic shock-induced decrease in mean arterial pressure.

#### Conclusion:

The results of this study have shown that the effect of hemorrhage on the decrease of mean arterial blood pressure, central vein pressure, blood acidity, and base excess is the same in the presence or absence of soft tissue injury. In this regard, we can say that soft tissue injury in the absence of hemorrhagic shock has no effect on hemodynamic and metabolic parameters of the blood.

#### Acknowledgments:

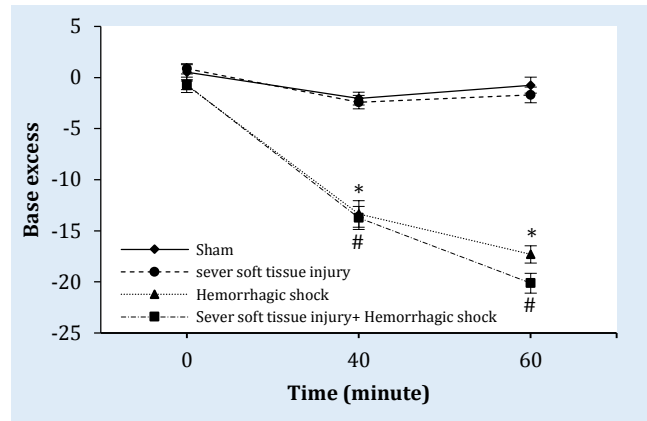
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#### Conflict of interest:

None

#### Funding support:

None



**Figure 4:** Comparison of base excess between the experimented groups. \*, shows significant difference of hemorrhagic shock group with the sham group at the same time. #, shows significant difference of severe soft tissue injury + hemorrhagic shock group with the sham group at the same time.

#### Authors' contributions:

All authors passed four criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editors.

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