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ORIGINAL RESEARCH

Subcutaneous Hematoma Elevates Plasma Levels of FDP and D-Dimer; an Analysis by Animal Model Experiments

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Purpose: Trauma-associated coagulopathy has been considered to develop as a result of increased fibrinolysis due to massive bleeding, tissue damage and hypoperfusion. However, it has not been investigated whether hematoma may cause trauma-associated coagulopathy. Using experimental animal model, we analyzed the effects of hematoma formation on coagulation and fibrinolysis parameters.

Materials: Male Wistar rats were used for the studies.

Methods: We made an animal model of subcutaneous hematoma without tissue injuries. This model can be categorised as a kind of trauma models. We created experimentally subcutaneous hematomas in test animals using blood collected from other animals. We performed blood sampling to measure blood cell counts and coagulation parameters from test animals at 1, 6, 24, 48 and 96 hours after hematoma generation. Blood samples were collected and immediately sent for measurement of CBC, Prothrombin, FDP, D-dimer, Fibrinogen, Antithrombin, AST and ALT. Furthermore, after 1, 24 and 48 hours, we performed dynamic evaluation of coagulation/fibrinolysis function using thromboelastometry method.

Results: After the hematoma were created, FDP and D-dimer increased over time, and reached a plateau after 48 hours. During the period, there was no decrease in Fibrinogen and Antithrombin, and no thrombocytopenia occurred. Moreover, no obvious changes in coagulation/fibrinolysis function were observed employing thromboelastometry.

Discussion: Elevated FDP and D-dimer after hematoma creation are assumed to be synthesized in the hematoma, not in the streaming blood. Thromboelastometry also shows that elevated levels of FDP and D-dimer are not caused by intravascular coagulation and subsequent fibrinolysis.

Conclusion: The study showed that subcutaneous hematomas caused increases in FDP and D-dimer levels, without activating the blood coagulation/fibrinolysis system.

Keywords: trauma, coagulopathy, fibrinolysis, intravascular coagulation

Introduction

Acute traumatic coagulopathy (ATC) is often seen among patients with severe injury and will lead to uncontrolled bleeding tendency, which contributes to trauma death.[1] The mechanisms for ATC are complicated. The possible mechanisms include activation of protein C, shedding of endothelial glycocalyx, release of catecholamine, dysfunction of platelets, acceleration of fibrinolysis, triggered by tissue injury and hypoperfusion. In the acute phase of trauma, tissue damage leads to coagulation activation, followed by fibrinolytic activation.[2] It is under intense discussion whether ATC is a new disease or just a disease entity similar to disseminated intravascular coagulation (DIC) with a fibrinolytic phenotype. Recently, coagulation abnormalities that occur after severe trauma have been named trauma-induced coagulopathy (TIC) and are recognized as a serious condition that can affect the life and death of trauma patients.[3]

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It has been considered that trauma injury accelerates fibrinolysis in early phase. The increase in levels of FDP and D-dimer after trauma has been recognized as evidence that hyper-fibrinolysis occurs.[4] Although thrombus formed in blood vessels after trauma causes secondary fibrinolysis, with an elevation in D-dimer level; nevertheless, it has not been proven sufficiently that FDP and D-dimer are derived from intravascular thrombus.

On the other hand, plasma FDP level is known to be high in patients with hemothorax or intraperitoneal hemorrhage, with or without trauma. Such conditions cannot be assessed as DIC. Blunt trauma not only causes hemorrhage into the soft tissue around the injured site but it also causes blood retention in the body cavity such as hemothorax and hemoperitoneum. Previous studies have not considered the impact of hematomas and intracavitary blood pools on assessing the post-traumatic changes in DIC parameters.

An animal model of subcutaneous hematoma was created to elucidate the effects of hematomas on the coagulation/fibrinolysis parameters. We analyzed the time-course changes in these parameters after hematoma creation and verified the effects of non-traumatic subcutaneous hematomas to the plasma levels of FDP and D-dimer.

Materials

We made an animal model of subcutaneous hematoma without tissue injuries. This model can be categorised as a kind of trauma models. Male Wistar rats of weight 280g were used for the experiments. We used 110 rats for laboratory tests before and after hematoma induction, including 10 rats for the control study. We used 50 rats for the recipient animals with hematoma (10 rats each in five groups for 1, 6, 24, 48, and 96 hours after inducing hematoma), and one donor rat was assigned to each of the recipient animals. Moreover, we used 21 rats for the dynamic measurement of coagulation function, including three rats for the control animals, nine rats for the hematoma recipient model (three rats each in three groups at 24, 48, and 96 hours), and nine rats for blood donor animals (three rats each in three groups at 24, 48, and 96 hours). Therefore, 131 rats were used in this experiment.

We are responsible for all aspects of the work in ensuring that questions related to the accuracy and integrity of all parts of the work are appropriately investigated and resolved. This study complies with the ethical regulation regarding experimental animals. All experiments follow the animal experimental guidelines as established by Kindai University and were approved by the ethical committee of Kindai University Hospital (Japan).

Methods

The Method for Blood Collection and Sampling

The experimental rat's abdomen was surgically cut open after administration of Isoflurane inhalation anesthesia, and 4 mL of blood was collected from the inferior vena cava.

The Method for Hematoma Creation

The 4 mL of blood collected from the donor rat was immediately injected subcutaneously on the back of the recipient rat, to create a subcutaneous hematoma.

Methods for the Laboratory Measurements

Blood was sampled from the control animals and the recipient rats at 24, 48, and 96 hours after inducing hematoma. Collected blood was used for the measurement of blood cell count, PT, FDP, D-dimer, Antithrombin, Fibrinogen, AST, ALT, and LDH. XT-1800i (Sysmex Company; Japan) was used to count the blood cell. Cobas-8000 (Roche-diagnostics; Swiss) was used for the measurement of AST, ALT, and LDH. C-2000 (Sekisui Medical Company; Japan) was used for the measurement of PT (%), FDP, Fibrinogen, and Antithrombin. Rat D-dimer ELISA kit (Kamiya Biochemical Company; Japan) was used for the measurement of D-dimer.

A second experimental line, where blood was sampled at 24, 48, and 96 hours after the hematoma creation for the dynamic measurement of coagulation/fibrinolytic function using the thromboelastogram method. ROTEMTM Thromboelastography (Tem Innovations GmbH; Germany) was used for this measurement.

Statistical Analysis

Time-course changes after hematoma creation in the measured data were determined using Kruskal–Wallis test, followed by DUNN's test as post-hoc test. The significancy was judged at $P < 0.05$.

Results

Photo of the experimental surgical site at 48 hours after inducing hematoma is shown in Figure 1. Clotted subcutaneous hematoma was observed on the back of the animal.

Changes in the counts of white blood cells and platelets over time after hematoma creation are shown in Figure 2. Changes in WBC statistically suggested that some of the groups' mean ranks consider to be different (H statistic 14.8273, degree of freedom 5, p-value 0.01113). However, no significant difference was observed between each group and the control group. Changes in Platelet counts statistically suggested that some of the groups' mean ranks consider to be different (H statistic 15.2294, degree of freedom 5, p-value 0.009426). However, no significant difference was observed between each group and the control group. Neither white blood cell counts nor platelet counts changed after hematoma creation. No changes were observed in CBC counts after hematoma creation.

Changes in FDP, D-dimer and antithrombin levels over time after hematoma creation are shown in Figures 3 and 4.

The plasma level of FDP was raised with time after hematoma formation and reached a plateau at 48 hours (Figure 3A). Changes in FDP were statistically suggested that some of the groups' mean ranks consider to be different (H statistic 44.0065, degree of freedom 5, p-value 2.309e-8). Significant differences were observed in the pairs of Control-48hr and Control-96hr.

D-dimer continued to rise over time and reached a plateau at 48 hours (Figure 3B). Changes in D-dimer were statistically suggested that some of the groups' mean ranks consider to be different (H statistic 48.4467, degree of freedom 5, p-value 2.879e-9). Significant differences were observed in the pairs of Control-48hr and Control-96hr.

Changes in antithrombin were shown in Figure 4. Changes in antithrombin were statistically suggested that some of the groups' mean ranks consider to be different (H statistic 31.2294, degree of freedom 5, p-value 0.000008439). However, no significant difference was observed between each group and the control group.

Figure 1 Photo of the experimental surgical site at 48 hours after hematoma creation. *There was observed a clotted subcutaneous hematoma.*

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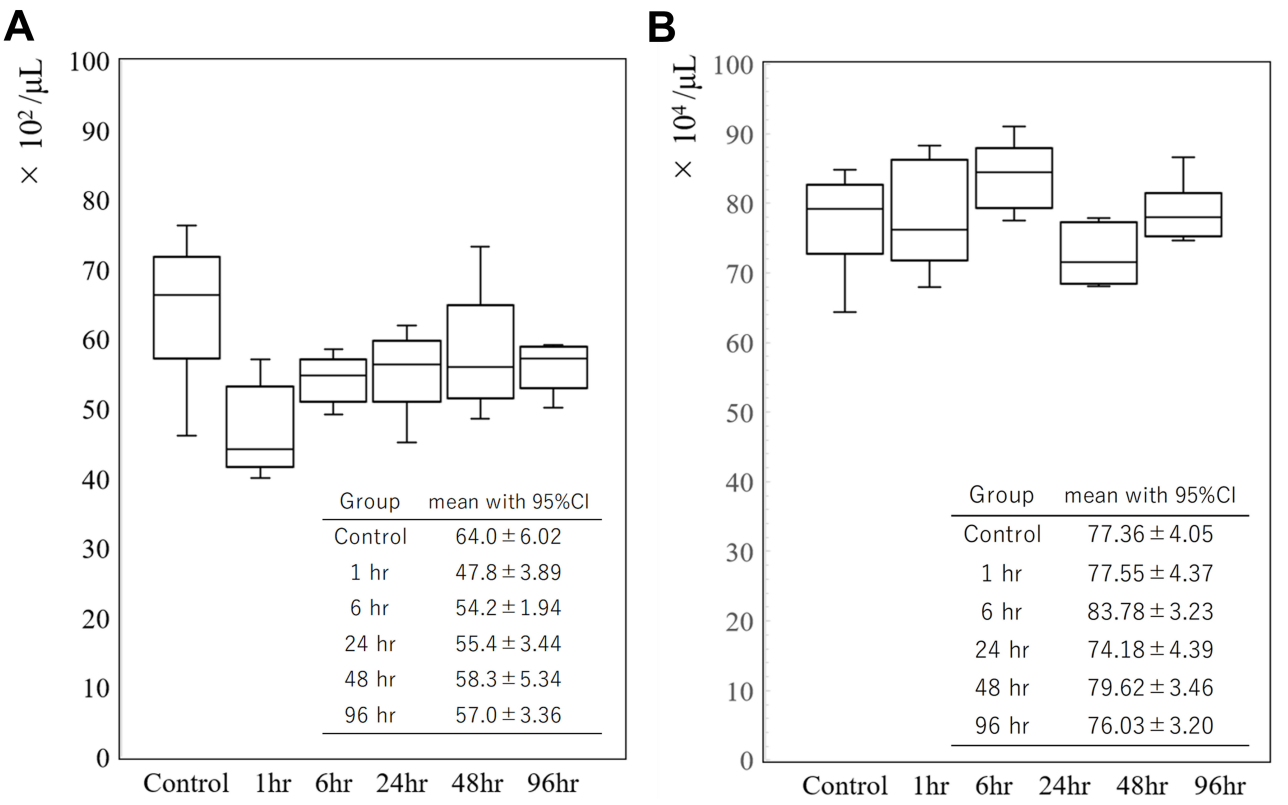

Figure 2 Changes in CBC count after hematoma creation. Data were presented with Box-and-whisker plots; the central box represents the values between the 10th and 90th percentiles, and the middle line is the median. Statistical significance was determined using Kruskal–Wallis test followed by DUNN's test. (**A**) Changes in WBC. *No change was observed after hematoma creation.* (**B**) Changes in Platelet count. *No change was observed after hematoma creation.*

Fibrinogen level did not decrease after hematoma creation after hematoma creation; data not shown. There were no changes in Prothrombin, AST, ALT, and LDH after hematoma creation; data not shown.

A graphic report of thromboelastometry after hematoma creation is shown in Figure 5. We did not observe any accelerations in coagulation/fibrinolytic activity after hematoma formation.

Discussion

In 1989, Kawakami et al investigated the coagulation parameters in patients with chronic subdural hematoma and reported that activation of both the clotting and fibrinolytic systems is associated with the development of the hematoma.[5] It has been known that a severely injured trauma patient often falls in a characteristic coagulopathy, which is associated with activation of coagulation, insufficient control of coagulation, fibrinolysis, and consumption coagulopathy.[1] Such conditions often lead to oozing type bleeding at mucosal lesions, serosal surfaces, and surgical-site wounds. Hemorrhagic shock associated with trauma causes tissue hypoperfusion and tissue hypoxia. Hypoxia promotes the vascular endothelium to release tissue plasmin activator, which causes DIC with enhanced fibrinolysis in the acute phase of trauma.[6–9] Usually, such conditions are medically controllable and improves rapidly with surgical hemostasis and replacement therapy. However, if the invasion is excessive, shock and acidosis will persist, resulting in DIC with suppressed fibrinolysis.[10]

Plasma levels of FDP and D-dimer are generally high in trauma patients, especially typical in cases with poor prognosis.[9–11] In blunt trauma, fibrinogen decreases early as a result of both coagulation activation and fibrinogenolytic activation. These pathological conditions result in DIC with enhanced fibrinolysis.[10,12–14] In severely traumatized cases, the coagulation system is enhanced immediately after the injury, followed by the acceleration of fibrinolytic system.[2,11] Many of these reports evaluate the post-traumatic elevations of FDP and D-dimer as a product of intravascular coagulation and subsequent fibrinolytic reactions.

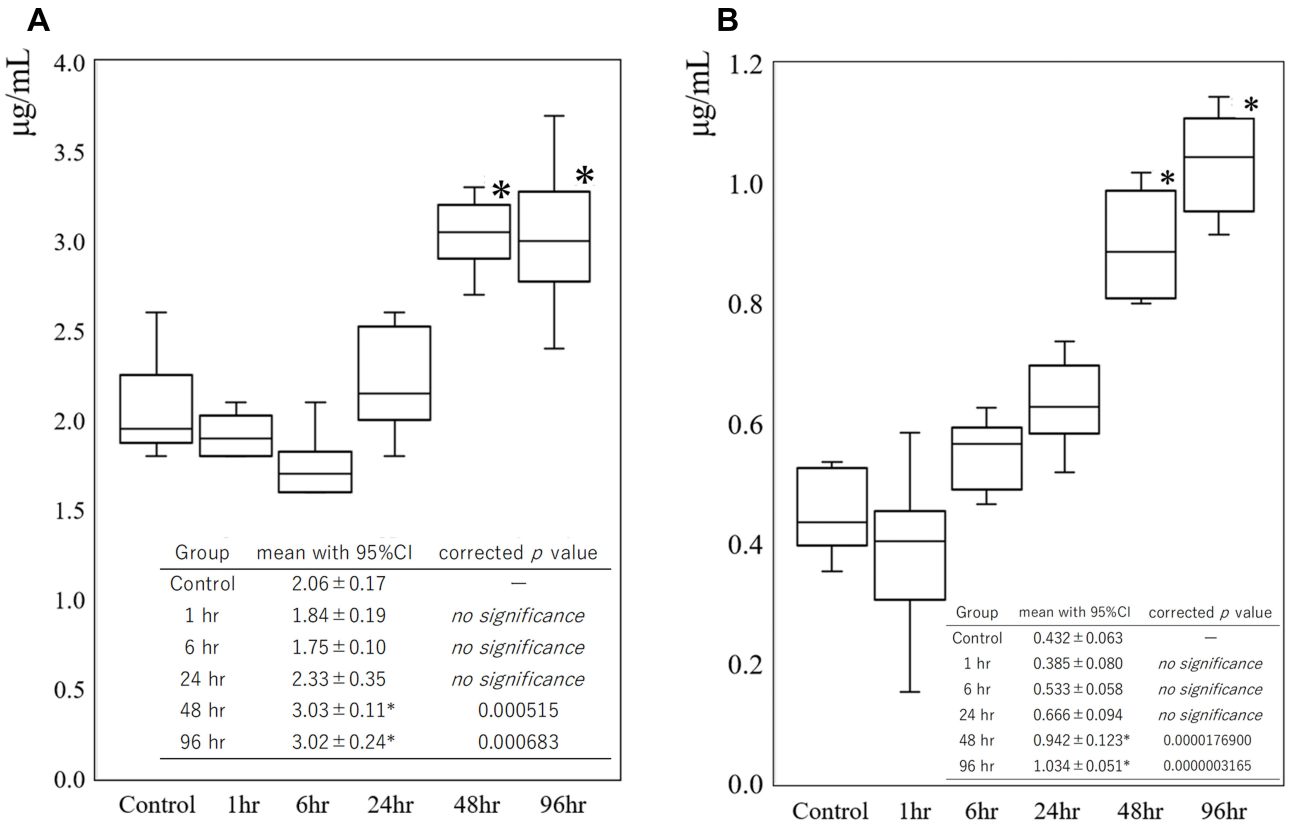

Figure 3 Changes in FDP & D-dimer level after hematoma creation. Data were presented with Box-and-whisker plots; the central box represents the values between the 10th and 90th percentiles, and the middle line is the median. Statistical significance was determined using Kruskal–Wallis test followed by DUNN's test. DUNN's test was applied to compare the data between each time group and the control group (*: $p<0.05$ compared with Control). (**A**) Changes in FDP level. *The FDP levels elevated after hematoma creation ($p<0.05$). Data of 48 and 96 hours were higher than the control (*: $p<0.05$).* (**B**) Changes in D-dimer level. *The D-dimer levels elevated after hematoma creation ($p<0.05$). Data of 48 and 96 hours were higher than the control (*: $p<0.05$).*

The concept of DIC is useful in evaluating the pathological condition of various coagulopathy. Two criteria have been applied commonly as tools for DIC diagnosis; one is provided by the International Society on Thrombosis and Haemostasis (ISTH), another is provided by the Japanese Association for Acute Medicine (JAAM). Both diagnostic criteria have adopted FDP as a scoring parameter and they can be applied for trauma cases.

We conducted animal experiments using a non-traumatic subcutaneous hematoma model, aiming to elucidate the effect of the presence of hematoma in the body on the patient's coagulation parameters, especially FDP and D-dimer. We analyzed changes in coagulation parameters over time after hematoma creation. While FDP and D-dimer were synthesized after the formation of hematomas, antithrombin and fibrinogen were not over-consumed. Our study demonstrated that non-traumatic subcutaneous hematoma elevated the plasma FDP and D-dimer levels, without affecting the function of the coagulation system.

FDP and D-dimer increased without consumption of fibrinogen and antithrombin, which are the precursor materials of FDP and D-dimers. We did not observe any dynamic changes in the function of coagulation and fibrinolysis system after hematoma formation. These facts suggest that the increased FDP and D-dimer were synthesized in extravascular hematoma.[13,15,16]

Physiological homeostasis constantly prevents blood clotting in blood vessels, while in the interstitial tissue, it quickly clots hemorrhaged blood out of blood vessels. Subcutaneously injected donor blood rapidly solidifies and forms a clot. This clot contracts while synthesizing fibrin. Rapid blood clotting and fibrin synthesis outside the blood vessels are caused by the abundant tissue factors there. Clot contraction also induces fibrinolysis within a clot.[17] We speculate that FDP and D-dimer synthesized here flow into the bloodstream.

Generally, the plasma levels of FDP and D-dimer are considered to reflect the intravascular coagulation and the subsequent fibrinolysis. These experiments proved that FDP and D-dimer levels increased in animals with hematoma and

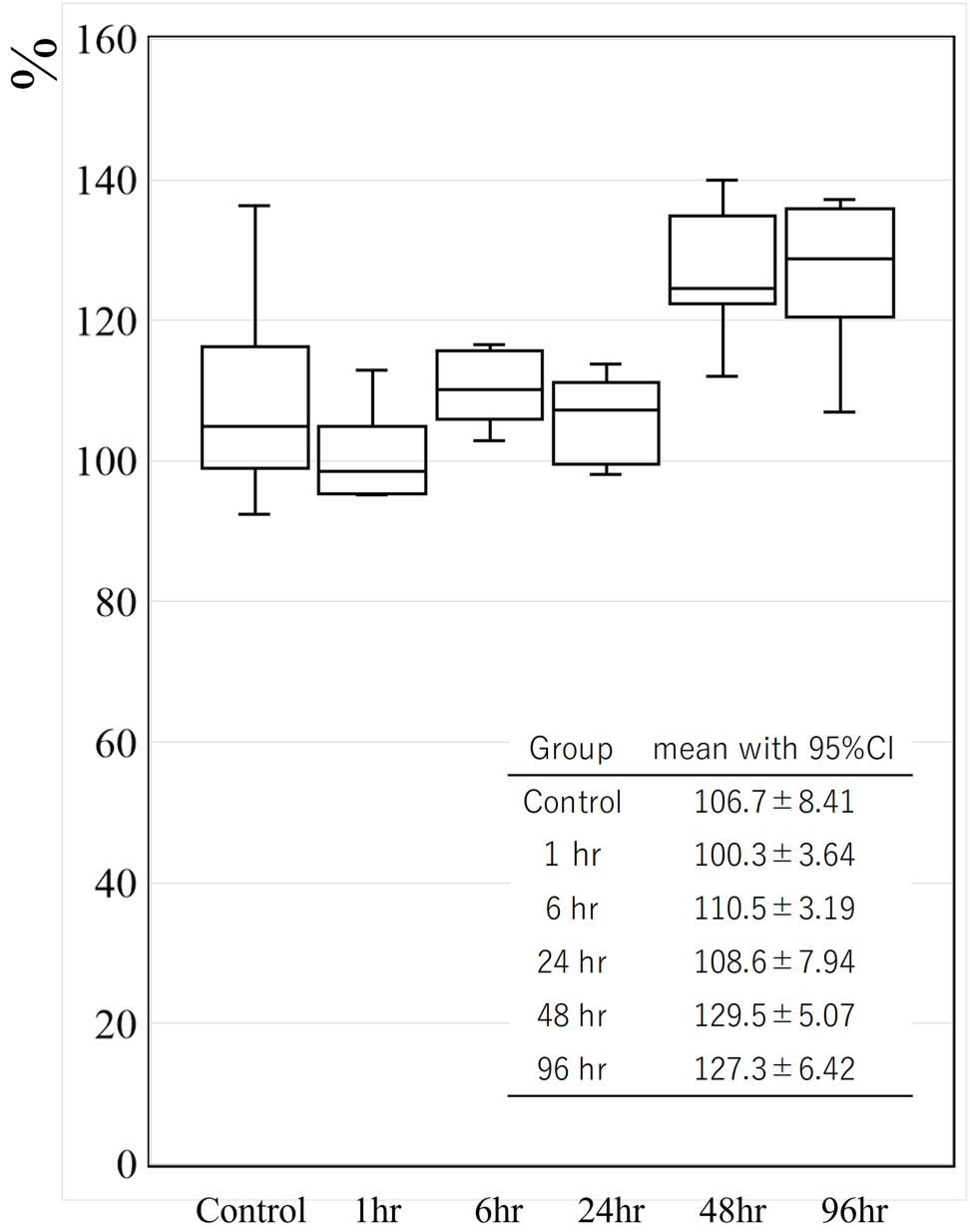

Figure 4 Changes in antithrombin level after hematoma creation. Data were presented with Box-and-whisker plots; the central box represents the values between the 10th and 90th percentiles, and the middle line is the median. Statistical significance was determined using Kruskal–Wallis test followed by DUNN's test as post-hoc test. *No specific changes were observed in Antithrombin level.*

that these products were derived from hematoma, not from intravascular coagulation. This may partly explain the elevated FDP and D-dimer in patients after trauma, especially in the cases with blunt injuries. Patients with hemothorax and intra-abdominal bleeding can also have a similar condition.

Most of blunt trauma patients must contain hematoma, to varying degrees, from extensive petechiae to subcutaneous hematoma or intra-coelomic hematoma, including hemothorax and hemoperitoneum. The hemorrhaged blood coagulates outside the blood vessels and forms hematoma, followed by fibrinolysis. In other words, it suggests that in blunt trauma patients, FDP and D-dimer are produced in the accompanying hematoma, and as a result, plasma levels in FDP and D-dimer elevate due to the fibrinolysis in the hematoma. Hematoma formation after blunt trauma can cause FDP and D-dimer elevations, even in the absence of tissue damage or hypoperfusion. In other words, elevated FDP and D-dimers do not directly imply intravascular coagulation in trauma patients.

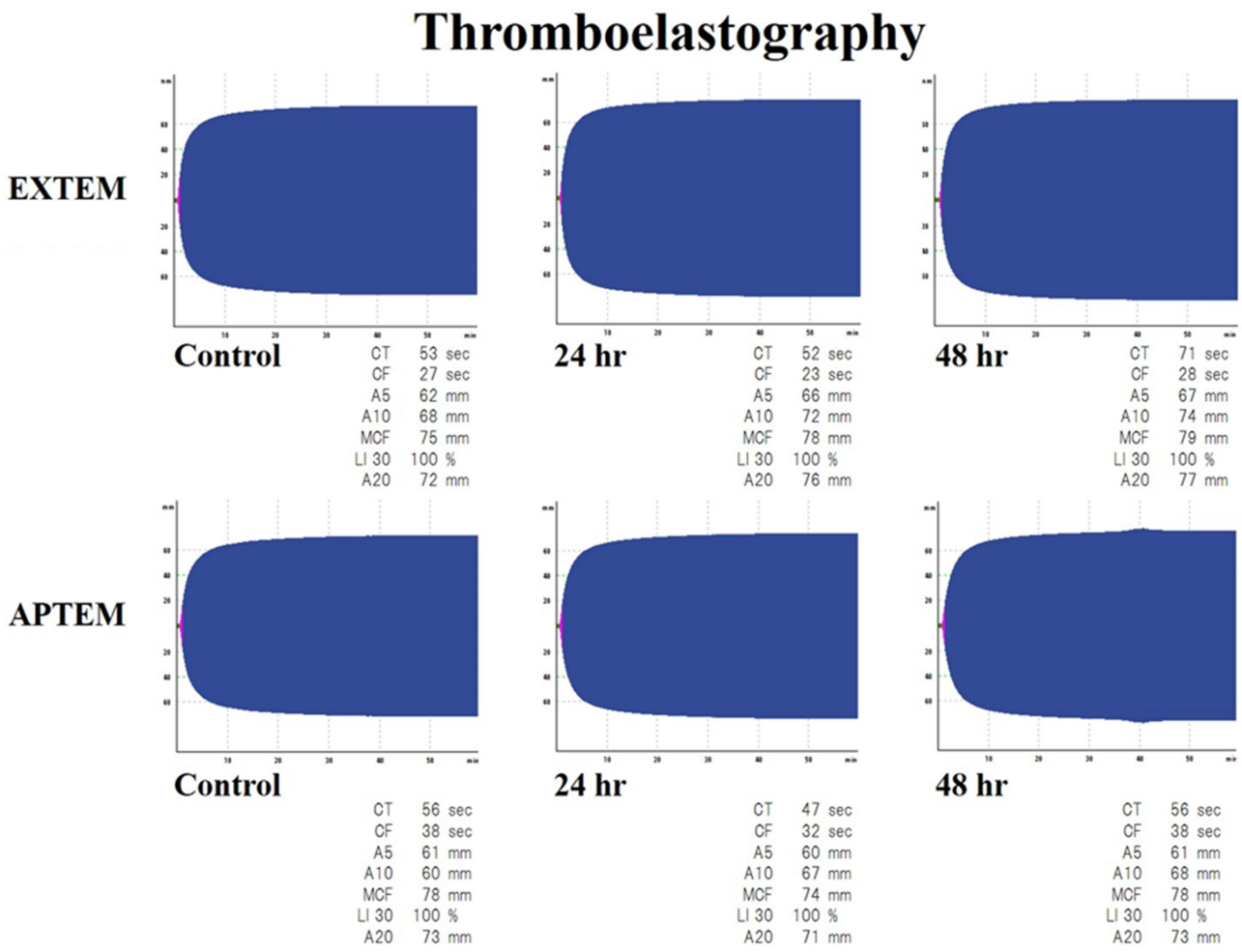

Figure 5 Graphic reports of thrombo-elastometry after hematoma creation. Any accelerations were not observed in coagulation or fibrinolytic activity after hematoma formation. EXTEM reflects dynamic activity in the external coagulation system. APTEM reflects dynamic activity of the fibrinolytic system.

In non-traumatic patients, FDP and D-dimer may also increase in such cases with marked subcutaneous hematoma or hemothorax. In these cases, we need to be careful in diagnosing DIC and not to overestimate FDP and D-dimer levels.

Conclusion

When assessing coagulation/fibrinolytic parameters in patients with suspected coagulopathy, it should be noted that elevated FDP and D-dimer do not necessarily mean intravascular coagulation, but sometimes include those derived from hematomas.

Disclosure

There is no conflict of interest in this manuscript, including financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest.

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