

The effect of pneumoperitoneum on intravascular fibrinolytic activity in rats

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Background/aim: Venous stasis during pneumoperitoneum in laparoscopic surgery is closely related to fibrin synthesis and deposition. The etiologic factors underlying fibrinolysis or hypercoagulability are not clearly defined. This study aimed to determine the effects of pneumoperitoneum time and pressure on coagulation cascade and the fibrinolytic pathway.

Materials and methods: After the pneumoperitoneum model was established in rats, PAI-1, tPA, TAFI, D-dimer, and fibrinogen activities were evaluated in different time periods under different pressures in groups including 6 rats. Group 1 did not undergo any procedure. Group 2 received 8 mmHg of pressure for 30 min, Group III 8 mmHg for 60 min, Group IV 12 mmHg for 30 min, and Group V 12 mmHg for 60 min.

Results: D-dimer levels had a tendency to decrease with increasing intraabdominal pressures. In both low and high pressure groups, fibrinogen had a tendency to increase with exposure time. There was no statistically significant difference among the study groups in terms of fibrinogen, D-dimer, and PAI-1. The levels of TAFI were significantly decreased with increasing pressure regardless of the exposure time.

Conclusion: Pneumoperitoneum of the coagulation system can be changed by duration of time and pressure.

Key words: Pneumoperitoneum, fibrinolysis, hypercoagulability

1. Introduction

Laparoscopy is widely used in modern surgical areas. It has common advantages in its favor, which have been summarized as rapid tissue recovery, less postoperative pain, less wound dehiscence, less hospitalization, and rapid return to work (1). Surgical trauma is known to effect hemostatic system functions adversely. This effect is particularly evident upon development of thromboembolic complications (2). Activation or inhibition of the coagulative cascade produces more complications intraoperatively or postoperatively. A tendency of postoperative thromboembolic complications results from two important changes in the coagulative system.

The tendency toward hypercoagulation is the first sign and the second changing parameter is an increase of fibrinolysis and a following decrease (3). Fewer complications are expected with laparoscopic surgery due to less damage to the tissues. However, effects of laparoscopic surgery on coagulation and the fibrinolytic system are not clear. Effects of pneumoperitoneum and

the reverse Trendelenburg position lead to a decrease of venous return and increase the predisposition to deep venous thrombosis (4–6). Less tissue damage, rapid wound healing, less surgical trauma, less operative time, and less immobilization time lead to a decrease in changes of fibrinolysis of 0.2%–2% (7,8).

Under normal conditions, fibrin production and breakdown depend on a tightly regulated control mechanism. When fibrinolytic activity predominates fibrin production, hyperfibrinolysis occurs, which results in bleeding disorders. tPA is released from the endothelial cells and mediates conversion of plasminogen to plasmin, whereas PAI-1 controls this pathway. Fibrin breakdown products, D-dimer, thrombin coagulation time, or thrombin time and thromboelastograms are the parameters used for the diagnosis of hyperfibrinolysis (9). TAFI cleaves lysine residues at the C-terminal of fibrin fragments, preventing plasminogen from binding to this site; therefore, it causes inhibition of the fibrinolytic activity (10).

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Is it possible to explain the effects of laparoscopic surgery on the hemostatic system by the duration of pneumoperitoneum or by pressure differences? In order to seek the answer to this question and to explain the effect of pneumoperitoneum on the fibrinolytic system, we used tPA, PAI-1, d-dimer, fibrinogen, and TAFI tests in this study. This study aimed to determine the effects of pneumoperitoneum time and pressure on coagulation cascade and the fibrinolytic pathway.

2. Materials and methods

2.1. Animal care and use

The study was approved by the local ethics committee and the animal experiments were performed according to the animal handling guidelines provided by the Gazi University animal laboratory. Thirty female Wistar albino rats weighing 300–350 g and aged an average of 4 months were used. The animals were kept at 21 ± 2 °C with 12-h cycles of light and dark. The animals were provided ad libitum rat chow and water. Anesthesia was performed with intramuscular 50 mg/kg ketamine hydrochloride (Ketalar; Parke Davis & Eczacıbaşı, İstanbul, Turkey) and 5 mg/kg xylazine hydrochloride (Rompun; Bayer Healthcare, Leverkusen, Germany).

2.2. Operative details and study groups

After administration of anesthesia the rats were fixed on a table. Their abdomens were shaved and cleaned with povidone iodine solution. An incision was made at the abdominal wall, approximately 5 mm long; one Veress needle (Ethicon Endo-Surgery, UV120, USA) was placed in the abdominal cavity. After its placement, the Veress needle was connected to an electronic insufflator (Storz and Co., Tutthufen, Germany), and carbon dioxide was insufflated until a steady pressure of 8 mmHg or 12 mmHg was maintained. The insufflator was set to readjust intraabdominal pressure by gas insufflation in the case of absorption of carbon dioxide gas by the peritoneal surface or any gas leakage.

In Group 1, a minilaparotomy was performed on rats, sterile Veress needles were placed in abdomens, and, without applying pneumoperitoneum, intracardiac blood samples were obtained at 30 and 60 min. The rats were then sacrificed. In Groups 2 and 3, pneumoperitoneum was applied to rats with 8 mmHg pressure as explained before for a duration of 30 min and 60 min, respectively, while in Groups 4 and 5 pneumoperitoneum was applied with 12 mmHg for 30 min and 60 min, respectively. Intracardiac blood samples were obtained at the end of these time periods and the rats were sacrificed.

Insufflation of CO₂ was arranged as continued administration to provide automatic constant intraabdominal pressure. Respiratory problems in rats that can occur due to both the CO₂ gas used for insufflation

and increased intraabdominal pressure were monitored via a pulse-oximeter placed on the cervical carotid artery noninvasively. Blood samples were taken from the inferior vena cava and rats were sacrificed through administration of intracardiac potassium under anesthesia. Laparotomy was then performed to identify any iatrogenic injury.

Group 1: Group in which blood samples were obtained at 30 and 60 min after a minilaparotomy was done and Veress needles were placed (sham), n = 6.

Group 2: Low pressure group with short time period. Pneumoperitoneum was created with 8 mmHg pressure and blood samples were taken after 30 min, n = 6.

Group 3: Low pressure group with long time period. Pneumoperitoneum was created with 8 mmHg pressure and blood samples were taken after 60 min, n = 6.

Group 4: Standard pressure group with short time period. Pneumoperitoneum was created with 12 mmHg pressure and blood samples were taken after 30 min, n = 6.

Group 5: Standard pressure group with long time period. Pneumoperitoneum was created with 12 mmHg pressure and blood samples were taken after 60 min, n = 6.

2.3. Blood tests

Blood samples were centrifuged at $3000 \times g$ in EDTA tubes for 10 min and serum samples were prepared. Serum samples were kept in Eppendorf tubes at -80 °C.

2.3.1. Measurement of serum fibrinogen level

Quantitative fibrinogen levels were measured by coagulometric method with a fibrinogen kit (American Diagnostica Inc.) and by the enzyme linked immunosorbent assay (ELISA) method according to the manufacturer's instructions. In this method a Tecan-8C64 brand washer and Tecan Sunrise brand reader machine were used by spectrophotometric method. Values were expressed as ng/dL.

2.3.2. Serum D-dimer level measurement

Quantitative D-dimer levels were measured by coagulometric method with a fibrinogen kit (American Diagnostica Inc.) and by the ELISA method according to the manufacturer's instructions. In this method a Tecan 8C64 brand washer and Tecan Sunrise brand reader machine were used by spectrophotometric method. Values were expressed as ng/dL.

2.3.3. Measurement of serum tissue plasminogen activator (tPA) level

Quantitative tPA levels were measured with the Imubind tPA kit (American Diagnostica Inc.) and by ELISA method according to the manufacturer's instructions. In this method a Tecan 8C64 brand washer and Tecan Sunrise brand reader machine were used by spectrophotometric method. Values were expressed as ng/dL.

2.3.4. Measurement of serum plasminogen activator inhibitor-1 (PAI-1) level

Quantitative plasminogen activator inhibitor-1 was measured with Imubind Plasma PAI-1 (American Diagnostica Inc.) and by ELISA method according to the manufacturer’s instructions. In this method a Tecan 8C64 brand washer and Tecan Sunrise brand reader machine were used by spectrophotometric method. Values were expressed as ng/dL.

2.3.5. Measurement of serum fibrinolysis inhibitor antigen activated by serum thrombin (TAFI)

Quantitative TAFI was measured with Imubind TAFI (American Diagnostica Inc.) and by ELISA method according to the manufacturer’s instructions. In this method a Tecan 8C64 brand washer and Tecan Sunrise brand reader machine were used by spectrophotometric method. Values were expressed as ng/dL.

2.4. Statistical analysis

Nonparametric tests were performed. Kruskal–Wallis variance analysis was performed to compare the five groups. Statistical results that were statistically significant were compared with the Mann–Whitney U Test. Operation time and intraabdominal pressure data were compared with the Mann–Whitney U Test. $P < 0.05$ was accepted as statistical significance. Median data are shown with minimum–maximum values.

3. Results

Rats were randomized into five groups as described in Section 2.2. The Table shows data of blood samples taken from rats: fibrinogen, D-dimer, PAI-1, tPA, and TAFI levels. Statistical results showed there was not any

significance statistically considering fibrinogen and D-dimer blood levels taken after a short or long time following pneumoperitoneum insufflated at different pressures ($P > 0.05$). PAI-1 levels in Group 2 were lower than in the 30-min sham group but there was no statistical significance ($P < 0.05$). PAI-1 levels were increasing with time and higher pressure but there was no significance statistically regarding time and pressure differences between groups ($P > 0.05$). tPA level was higher in Group 4 but in Group 5 it was low. This difference between the two groups was significant statistically ($P = 0.04$). TAFI levels showed a decreasing trend in all groups; in Group 5, this decrease was statistically significant ($P < 0.05$) (Table). There was no significance statistically between groups regarding fibrinogen, D-dimer, and PAI-1 levels ($P > 0.05$); however, tPA and TAFI levels between groups were significant statistically and this shows activation of the fibrinolytic process ($P < 0.05$ and $P < 0.01$).

4. Discussion

Important hemorrhagic and thrombotic processes begin clinically after degradation of fibrin deposits by an organism (11,12). The fibrinolytic system is important in order to understand this complex processes. There are still some unknown physiopathological processes of fibrinolytic activators after trauma. Postoperative changes in fibrinolytic activity can lead to thromboembolic events and hemorrhage (13).

Postoperative thromboembolic event and intraoperative hemorrhage are important complications after surgical abdominal procedures. Pneumoperitoneum and venous stasis of the lower extremities due to laparoscopic procedures could lead to these complications

Table. Comparison of parameters among groups.

Parameters	Median(min–max)						P*
	Group 1, 30 min	Group 1, 60 min	Group 2	Group 3	Group 4	Group 5	
Fibrinogen	203 (82–381)	204 (87–381)	123 (70–270)	164.5 (119–386)	127.5 (70–190)	148.5 (94–250)	0.22
D-dimer	0.190 (0.190–0.450)	0.190 (0.190–0.450)	0.195 (0.100–0.450)	0.190 (0.190–0.300)	0.195 (0.190–0.350)	0.190 (0.190–0.200)	0.68
PAI	1.029 (0.303–2.465)	1.042 (0.362–2.395)	0.829 (0.427–1.105)	0.974 (0.846–1.765)	1.088 (0.780–3.520)	1.254 (0.358–2.341)	0.58
TPA	1.335 (0.415–2.359)	1.328 (0.417–2.263)	0.959 (0.700–2.109)	1.214 (0.047–3.809)	1.775 (0.446–7.873)	0.402 (0.343–0.514)	0.04
TAFI	21.886 (9.374–28.924)	18.457 (9.013–21.716)	13.240 (10.576–16.358)	12.389 (11.151–18.146)	11.698 (7.579–14.770)	2.718 (2.555–3.901)	0.01

*: Kruskal–Wallis test.

(14); however, some studies showed reverse findings (15,16).

Nevertheless, pneumoperitoneum leads to anoxia in endothelial cells and increases in endothelial cell-related tPA levels in plasma, and so thromboembolic events can form. Studies showed that 12–14 mmHg of intraabdominal pressure over 60 min leads to damage to venous endothelial cells in the anterior abdominal wall. Due to damage of endothelial cells, plasma tPA level increases gradually (17). Studies do not clarify the etiological relationships between pneumoperitoneum time and gas pressure values, fibrinolysis, and postoperative thromboembolic events. This is because there are limited data about the effect of duration of pneumoperitoneum and adequate gas pressure to constitute pneumoperitoneum.

In this study our aim is to clarify the etiological relationship between pneumoperitoneum and coagulation cascades, so fibrinolytic parameters were studied and these parameters were analyzed considering the duration of pneumoperitoneum and differences in gas pressure.

Dexter et al. studied tPA, PAI-1, D-dimer, and euglobulin clot lysis time levels during laparoscopic and open cholecystectomy (18). It was shown that PAI-1 levels were higher in laparoscopic cholecystectomy at the postoperative 6th hour; however, other parameters did not show any difference. Another study compared laparoscopic and open cholecystectomy in terms of tPA, plasmin antiplasmin, and PAI-1 levels. Results did not show any difference between two groups perioperatively (19). Van der Velpen et al. studied fibrinogen, tPA, and PAI-1 levels nonrandomized during laparoscopic and open cholecystectomy, and the groups showed differences in their results (20).

Another randomized study compared laparoscopic and open cholecystectomy in terms of fibrinogen, prothrombin fragment F1 + 2, D-dimer, and PAI-1 levels, which did not show any significance statistically (15). However, Caprini et al. compared these two surgeries with thromboelastographic indexes and a hypercoagulable state was reported in favor of laparoscopic cholecystectomy (21). Schietroma et al. showed high fibrinogen levels at the postoperative 72nd hour of patients who had undergone laparoscopic cholecystectomy. Pneumoperitoneum formed under 14 mmHg and mean operation time was 40 min. Additionally, plasminogen levels were decreased while fibrinogen levels were gradually increasing. These data are important in order to explain the relationship between pneumoperitoneum and fibrinolysis. Another study showed lower fibrinogen levels after laparoscopic surgery than after open surgery (22). In the same way, fibrinogen levels at the postoperative 1st hour decreased but postoperative 24th, 48th, and 72nd hour levels were gradually increased (23). As an acute phase reactant

fibrinogen tends to be increased after any trauma. It can be concluded that high fibrinogen levels in this study can be related to surgical trauma.

D-dimer level is usually used as an indicator of intravascular clot formation (24). Its level can increase as a result of fibrin degradation after trauma or in postoperative terms. Neudecker et al. studied tPA, PAI-1, tPA/PAI-1 complex, fibrinogen, and D-dimer levels in patients undergoing laparoscopic and open colorectal resections (22). That study did not show statistical significance between the two groups. Nguyen et al. studied TAT, F1 + 2, fibrinogen, D-dimer, AT III, and protein C levels in patients undergoing laparoscopic and open gastric banding surgery (25).

The pivotal enzyme of the fibrinolytic cascade is plasmin. Plasmin is formed by activation of plasminogen. Activation of tPA is regulated by PAI-1 (26,27). Animal studies on active inhibitor PAI-1 showed that high levels of PAI-1 are effective in preventing arterial and venous thrombosis. Other human and animal studies showed that low levels of PAI-1 lead to increased bleeding. The PAI-1 level increases in the early period after trauma or surgical stress but decreases later. These studies finally showed that trauma and surgical stress can affect the degree of thrombosis and bleeding (28).

In our study, levels of fibrinogen, D-dimer, and PAI-1 were compared and results did not show any significant difference. Literature findings showed the results of pneumoperitoneum and surgical trauma together, so this could explain the different results of our study. However, the results of our study showed the effect of duration of pneumoperitoneum and change of intraabdominal pressure in laparoscopic surgery on the intravascular fibrinolytic system and it can be excluded that another impact besides the stress of these parameters on fibrinolysis could be neglected. Protection of the organism from arterial and venous thrombosis could be assured by removal of fibrin deposits, which means, in general, fibrinolysis. The pivotal enzyme of fibrinolysis is plasmin and it is formed by plasminogen via tPA. In the absence of tPA microvascular thrombosis and microvascular fibrin deposits are indispensable. Without fibrin deposit tPA cannot activate plasminogen effectively. In the presence of fibrin it is a strong activator. Studies that examined the effects of laparoscopic and open surgery on the fibrinolytic system showed that activation of the effect of PAI-1 independently results in the decrease of tPA levels; however, this result was not found to be statistically significant (29,30). Our study showed low tPA levels in Group 5, in which pneumoperitoneum was formed by 12 mmHg for 60 min ($P < 0.05$). There was no significance statistically among other groups in terms of tPA levels ($P > 0.05$). This result can be interpreted such that the fibrinolytic cascade

was activated after cessation of pneumoperitoneum in Group 5. This result could be due not only to duration of pneumoperitoneum but also pressure difference. Reduced tPA in this group suggests that the coagulation system is shifted towards fibrinolysis, which is also supported by low TAFI values in the same group. However, in contrast with the results of other studies in the literature (13,31), these results indicate that coagulation system is shifted toward the fibrinolytic system. One reason for this condition is that our study is different from the other studies previously conducted in this area since CO₂ gas was used at standard pressure (12 mmHg) and low pressure (8 mmHg). In other studies, pressure was adjusted to maintain 12–14 mmHg as the standard. The effect of pneumoperitoneum occurs due to venous stasis particularly when high pressure is used. Utilization of standard and low pressures in our study might have caused different results than reported

previously. Additionally, in many studies, the coagulation system was shown to shift in favor of thrombosis at 6 and 24 h after termination of pneumoperitoneum. However, blood samples were taken immediately after termination of pneumoperitoneum in our study. For this reason, acute effects of pneumoperitoneum may include reduction in tPA and TAFI.

In conclusion, this study reports effects of pneumoperitoneum on the coagulation system, which can be changed by duration of time and pressure. Even at standard pressure for pneumoperitoneum formation, increased time can lead to complications related to fibrinolysis in the acute period. Another future step for study could involve higher pressure levels and extended operation times. Additionally, more studies should be planned to study the effects of pneumoperitoneum on the coagulation system.

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