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ORIGINAL ARTICLE

The Effect of Carbon Dioxide Insufflation Applied at Different Pressures and Periods on Thrombotic Factors

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Abstract The aim of this experimantal study which is applied on rats, is to determine the differences on the clotting factors over the application of low and high intraabdominal pressure (IAP) values in different periods of time in carbon dioxide (CO_2) pneumoperitoneum. Thirty rats were randomized into five groups (n = 6): a control group (Group K) and 1 h and 6 mm Hg IAP (Group A), 2 h and 6 mm Hg IAP (Group B), 1 h and 12 mm Hg IAP (Group C) and 2 h and 12 mm Hg IAP were created with CO_2 pneumoperitoneum (Group D). At the end of the experiment, plasma samples taken from subjects and fibrinogen, FII (prothrombin), FV, FVII, FVIII, FIX, FX, FXI, FXII, von willebrand's factor (vWF), ristocetin cofactor, protein C, protein S, antithrombin III (AT III) levels are studied. There were statistically significant differences in the mean levels of FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, and protein S between the groups. A hypercoagulable state occurred with the following: increase in the coagulation parameters compared to the control group; increase in FVII in the group only Group C; decrease in AT III in all groups compared to the control group; decrease in protein C in the group only XII Group D compared to control group; decrease in protein S in all groups except group D compared to control group. CO_2 insufflation predisposes to thromboembolic events both by inducing coagulation factors and by suppressing the fibrinolytic system contrary to the controversies in the literature.

Keywords Pneumoperitoneum · Carbon dioxide · Rats · Coagulation parameters

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Introduction

It is a well known fact that trauma caused by open surgical procedures is a predisposing factor for thrombotic events. Less thrombo-embolic complications in laparoscopic surgery causing less tissue trauma is expected. Increase in the incidence of venous thromboembolism (VTE) after laparoscopic surgical procedures accompanied by pneumoperitoneal pressure (PP) in the recent years directed the researchers towards this subject [1].

Many experimental and clinical studies were performed due to the increase in VTE complications in the patients undergoing laparoscopic surgery and these studies especially focused on PP. Carbon dioxide (CO_2) is the most frequently used gas for insufflation into the peritoneal cavity for providing a sufficient field of view during laparoscopic surgery. Increased intraabdominal pressure (IAP), increased peritoneal CO_2 absorption and patient positioning during surgery were determined to cause some hemodynamic changes [1]. While an increase occurs in systemic vascular resistance, CVP and intrathoracic pressure, a decrease occurs in renal, portal blood flow and lung compliance. Marked hypercapnia and acidosis may occur due to unfavorable effects on respiratory functions caused by IAP increase together with absorption of CO_2 through the peritoneal surface. Laparoscopic surgery causes a kind of ischemia–reperfusion injury following recovery of reduced splanchnic circulation after desufflation [2]. Increase

in IAP caused by PP leads to an increase in venous blood pressures while it results in reduction of vena cava inferior flow and venous return in lower extremities [3]. Also, reduced splanchnic circulation improves after desufflation and reperfusion injury occurred in vessel endothelium initiates thrombus formation [3, 4].

In an experimental study performed in the rats, it was aimed to demonstrate the changes on the coagulation factors caused by administration of low and high IAP values in various periods in CO_2 pneumoperitoneum.

Materials and Methods

Thirty female adult Wistar Albino rats (200–240 g) were used in the study. Five groups each including six rats were formed according IAP created by CO_2 PP and duration of administration variables which will be administered.

Before surgical procedure, all rats were anesthetized by an intramuscular (im) Ketamine Hydrochloride 50 mg/kg and im Xylazine hydrochloride 5 mg/kg.

Group K: Veress needle was inserted into the peritoneal cavity in the midline between xiphoid and symphysis pubis and then removed.

Group A: Veress needle was inserted into the peritoneal cavity. Insufflation was performed at the rate of 0.2 L/min as IAP to be 6 mmHg for PP. IAP was kept at this level for 1 h with automatic insufflation device (Karl Storz, Germany). After 1 h, desufflation was performed and allowed for 1 h for reperfusion.

Group B: PP was produced as IAP to be 6 mmHg and kept at this level for 2 h.

Group C: PP was created as IAP to be 12 mmHg and kept at this level for 1 h.

Group D: PPP was created as IAP to be 12 mmHg and kept at this level for 2 h.

Following 1 h reperfusion period, blood samples were taken and the animals were sacrificed in all groups.

Blood samples were centrifuged twice at 3500 rpm and plasma was separated. During the period for sample collection, plasma was frozen at −80 °C and stored. Except the subject number five in group D (due to coagulation), fibrinogen, FII (prothrombin), FV, FVII, FVIII, FIX, FX, FXI, FXII, von willebrand's factor (vWF), ristocetin cofactor (RCOF), Protein C, Protein S and antithrombin III (AT III) values of all other plasma samples were determined using spectrophotometric method on the analyzer (ACL Top 700 automated coagulometer, Beckman Coulter Inc. US).

D-Dimer and protein C were compared by Kruskal–Wallis. Other parameters levels were compared by one-way ANOVA. A p value <0.05 was considered significant.

Results

In all four groups in which CO_2 PP was applied at different periods and pressures, a hypercoagulable state occurred with the following: increase in the coagulation parameters like fibrinogen, FII, FV, FVIII, FIX, FX, FXI, FXII, WF and RCOF compared to the control group; increase in FVII in the group only 12 mmHg pressure was applied for 1 h (Group C); decrease in ATIII in all groups compared to the control group; decrease in protein C in the group only 12 mmHg pressure was applied for 2 h (Group D) compared to control group; decrease in protein S in all groups except group D compared to control group (Table 1).

Discussion

The superiority of laparoscopy over the open procedure was currently demonstrated with the following advantages like early mobilization, less tissue injury, short hospitalization period, less pain and inflammatory process, reduced surgical stress. Percent of the complications like deep vein thrombosis (DVT) and pulmonary embolism are less than the open surgery as a result of early mobilization and less tissue injury. With the reports of rare complications like portal vein thrombosis even after surgeries requiring relatively short period of time like gallbladder surgery in which laparoscopy used commonly, studies investigating the effects of CO_2 insufflation on the coagulation parameters were started.

Animal studies showed that subendothelium might be exposed to coagulation and thrombocyte activation due to injury occurring in the vessel wall after PP and increased IAP might have caused damage in the synthesis of vitamin K dependent coagulation factors by affecting hepatic circulation and biliary excretion [5]. The role of stress caused by stasis and increased pressure developing due to reduction in venous return on endothelial injury was emphasized [3]. Some authors attributed hyper-coagulability occurring after the surgery directly to vasospasm rather than coagulation factor disorders. It was stated that endothelin was also secreted in addition to coagulation activation with endothelial injury of the vessel and vasospasm developed and the complications like DVT were increased [6]. In the light of prospective studies, asymptomatic DVT was determined at a rate of 23–55 % after laparoscopic cholecystectomy (LC) [7, 8]. Increased splanchnic vessel thrombosis after laparoscopy was attributed to hemodynamic effects of CO_2 insufflation. Increased IAP due to CO_2 insufflation and hypercapnia occurred increase the peripheral resistance, arterial, pulmonary and pulmonary capillary wedge pressure by causing sympathetic vasoconstriction. With effects of these changes, cardiac index

Table 1 Coagulation and anticoagulation factors levels of groups (mean ± SD)

Groups	A	B	C	D	K	p value
Fibrinojen n (mg/dl)	125.83 ± 9.54	139.67 ± 54.33	131.67 ± 24.17	134.60 ± 12.12	124.00 ± 31.53	0.909
FII (%)	64.67 ± 3.27	66.67 ± 6.06	75.67 ± 8.21	63.60 ± 3.78	62.33 ± 3.88	0.002
FV (%)	64.00 ± 5.51	66.83 ± 7.70	78.33 ± 5.68	62.20 ± 4.38	60.17 ± 5.49	0.000
FVII (%)	67.50 ± 4.51	69.83 ± 12.91	78.33 ± 4.89	63.60 ± 7.16	71.33 ± 0.82	0.032
FVIII (%)	62.33 ± 4.50	73.33 ± 5.13	79.50 ± 8.78	56.00 ± 4.74	55.17 ± 2.56	0.000
FIX (%)	60.67 ± 6.74	70.17 ± 5.98	77.83 ± 8.59	55.80 ± 4.92	54.17 ± 4.67	0.000
FX (%)	71.00 ± 7.85	70.83 ± 9.04	82.83 ± 5.98	64.80 ± 7.79	68.83 ± 7.19	0.008
FXI (%)	63.17 ± 5.81	67.83 ± 7.99	76.50 ± 7.34	60.00 ± 7.52	57.33 ± 4.13	0.000
FXII (%)	64.33 ± 3.33	68.00 ± 6.26	80.00 ± 11.44	62.60 ± 4.98	57.83 ± 6.15	0.000
D-Dimer (ng/ml)	22.67 ± 12.34	29.33 ± 16.68	22.50 ± 28.26	10.40 ± 6.35	22.17 ± 20.86	0.072
vWF (%)	64.83 ± 5.15	72.33 ± 8.87	86.50 ± 17.55	83.00 ± 37.64	62.83 ± 13.11	0.158
RCOF (%)	63.83 ± 8.28	69.00 ± 8.00	83.33 ± 5.79	83.40 ± 44.94	61.50 ± 9.79	0.202
Protein C (%)	4.50 ± 1.64	5.83 ± 8.47	5.17 ± 1.33	3.40 ± 1.52	3.67 ± 1.51	0.228
Protein S (%)	1.25 ± 0.61	1.67 ± 0.52	6.50 ± 7.42	5.40 ± 3.05	7.67 ± 3.93	0.036
ATIII (%)	136.00 ± 11.47	134.83 ± 14.39	141.00 ± 14.09	125.20 ± 16.22	148.67 ± 11.0	0.093

decreases, portal venous blood flow may decrease by 70 % and stasis occurs [9, 10].

In Topal et al. experimental study in rats, they have observed that the tendency toward coagulation in the group exposed to high PP was greater than that of the other groups. This may be due to the fact that high intra-abdominal pressure causes more stasis in blood flow or high intra-abdominal pressure leads to more tumor necrosis factor, interleukin etc. release [3]. In laparoscopic surgery, the PP reduces venous reflux from the lower extremities by approximately 30–40 %. Therefore, the risk of DVT might be increased after laparoscopic surgery [11].

Schictroma et al. investigated cytokine, coagulation and fibrinolysis parameters at various hours before and after open cholecystectomy and LC. While plasma ATIII, protein C and plasminogen values were found to be reduced in both groups; F1-F2, thrombin-antithrombin, fibrinogen, fibrin and D-Dimer values were increased in favor of open surgery. Consequently, although to a lesser extent than the open surgery, medium hypercoagulability was found to be more significant in open surgery as determined also in laparoscopic surgery [12]. Also in our study, ATIII and protein C values were decreased compared to the control group while fibrinogen value was increased. No significant difference occurred regarding D-Dimer value. Similarly, after LC, Prisco et al. demonstrated significant increases in prothrombin fragments (F1+F2) and Vecchio et al. demonstrated significant increases in β-Thromboglobuline, PT, fibrinogen and D-Dimer values but these values were determined to be lower than the open surgery. These hypercoagulable results were attributed to high affinity of β-Thromboglobulin released from α granules due to thrombocyte activation and protein factor 4 to heparan sulfate in the endothelial membrane and denaturation in the membrane [13, 14]. In our study, fibrinogen values were determined to be higher than the control group, while significant difference was not observed in D-Dimer parameter. TAT, fibrinogen, D-Dimer parameters were increased in both LC and open cholecystectomy, but this increase was found to be more significant in open surgery in prospective randomized study performed by Tsiminikakis et al. [15]. In our study, a significant difference was not determined regarding D-Dimer, while fibrinogen values which were higher than the control group caused a hyper-coagulable state. In a patient who was normal before the surgery and then undergone laparoscopic sigmoid colectomy for diverticulitis, portal vein thrombosis was reported following surgery and protein S value was found to be lower [4]. Also in our study, a statistically significant decrease occurred in protein S values ($p < 0.05$). In addition to aforementioned studies, in the study performed by Larsen comparing gasless and conventional LC's, while an increase occurred in F1+F2 and D-Dimer parameters in both group, a statistically significant difference was not observed between the groups. In the study performed by Milic comparing open cholecystectomy and LC, a significant difference was not determined between the groups regarding ATIII, FVII, D-Dimer values. The prominent points in this study are the decrease in FVII values which is associated with increase in ATIII and D-Dimer values and evident in especially 13 patients developing DVT [16, 17]. In our study, a significant increase was observed in FVII values together with decrease in ATIII values compared to the control group. In another study, while a decrease was determined in protein S, protein C and ATIII parameters together with increase in fibrinogen values in the venous

blood samples taken from the upper and lower extremities of 25 patients undergone LC similar to our study, a statistically significant difference was not observed between the groups [18]. Martinez-Ramos stated that the coagulation parameters were not increased after LC; on the contrary, LC did not have anti-thrombotic effect by determining increase in plasma fibrinolytic activity and euglobuline fibrinolytic activity and D-Dimer value [1].

These studies investigating the effects of CO_2 insufflation on coagulation parameters were the ones performed generally in the patients and frequently after the surgeries like LC and colectomy. Therefore, factors like patient age, gender, comorbid factors of the patient, weight, operation time, tissue injuries caused by surgical procedure, anesthesia and fluids-medications administered, reverse Trendelenburg position, investigation of the parameters in different periods of time, pain thresholds and patient mobilization are effective on the results of studies. Although none of the aforementioned studies gives an opinion about the effects of CO_2 insufflation alone on the coagulation parameters, it seems to be impossible to perform such a study on the patients. Because of this, it was aimed to show the effects of CO_2 insufflation on the coagulation parameters of rats which was applied at different periods and pressures by planning experimental study and constituting 4 different groups compared to the control group. Differently from the studies in the literature, all factors of coagulation and fibrinolytic systems were investigated. This experimental study was worthy since there was no such a study performed in the literature investigating the effects of CO_2 insufflation on 15 different coagulation and fibrinolytic system parameters beginning from fibrinogen to ATIII which was applied at different periods and pressures.

We investigated coagulation and fibrinolysis parameters after 1 h of insufflation. Increased MDA levels of the lung homogenates at 2 and 6 h and control levels at 18 h. Almost no difference in protein carbonyl content. Decreased reduced glutathione levels in the lung tissue at 2 h after laparoscopy with control levels at 18 h after laparoscopy in rats [19]. Vecchio et al. evaluated the coagulation pathway assessing prothrombin time, activated partial thromboplastin time, prothrombin fragment F1 + 2, fibrinogen, and antithrombin III activity during LC in human. They also investigated fibrinolysis by determining plasma D-dimer concentration and platelet activation by measuring β-thromboglobulin. Twenty four hours after surgery there was a significant increase of fibrinogen, D-dimer, and beta-thromboglobulin and a slight increase of prothrombin time. They were not able to demonstrate changes of the other coagulation parameters. Probably these parameters could have increased earlier after insufflation and returned to normal at 24 h [14, 20].

In this study, the coagulation parameters like fibrinogen, FII, FV, FVIII, FIX, FX (except group D), FXI, FXII, WF and RCOF respectively caused a hyper-coagulable state by increasing in 4 groups in which CO_2 insufflation was applied at different periods and pressures compared to the control group. FVII value was increased only in rats which 12 mmHg pressure applied for 1 h (Group C) and was normal in other groups. Furthermore, while reaching the aforementioned parameters except fibrinogen and RCOF to the statistical peak values in group C is dependent on the induction of these parameters in increased IAP much more and investigation of the blood levels after reaching the peak values, presence of lower values in group D in which same pressure and long-term CO_2 insufflation is applied compared to group C can be attributed to approximation of the factors to the normal at the end of extended period of time. When considered for fibrinolytic activity, D-Dimer value was less in all groups except group A compared to the control group (lowest in group D), while protein C value was observed to be low in group D compared to the control group. D-dimer can be considered a predictor of thromboembolism and intravascular clot formation. D-dimer is a cross-linked fibrin degradation product, which forms as a result of a breakdown of fibrin. D-dimer levels are frequently increased after surgery or trauma and indicate the presence of an intravascular clot that has undergone lysis. D-dimer levels were elevated in open and laparoscopic cholesytectomy, but significantly less so after laparoscopic than after open cholecystectomy at 1 and 24 h. It can be considered a predictor of thromboembolism if its value exceeds a threshold of 1200 ng/mL. Vecchio et al. did not have any clinical evidence of deep vein thrombosis, although in four patients D-dimer was higher than 500 ng/mL. Mild hypercoagulability was observed in patients who had undergone LC without thrombophilia. This informations may be evidence that at the subclinical level there is less intravascular clot formation with a laparoscopic than with a traditional surgical insult [12, 14, 21]. Protein S value was determined to be low in all groups except group D compared to the control group. ATIII value was determined to be low in all groups compared to the control group (lowest in group D). Although these low levels are not statistically significant, they represent a pathological state. Together with these results, it can be stated that degradation of clot-thrombus occurring as a result of decrease in the fibrinolytic activity is delayed and this condition may predispose to thromboembolic events in all groups except protein C and especially in the group in which CO_2 insufflation is applied at high pressure and long period.

In conclusion, this experiment showed that CO_2 insufflation predisposes to thromboembolic events both by inducing coagulation factors and by suppressing the fibrinolytic system contrary to the controversies in the literature. This state is more evident in the conditions in

which CO_2 insufflation is applied at high pressure and long period.

Conflict of interest No financial interest/relationships with financial interest relating to the topic of this article have been declared.

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