Perioperative effects of anesthesia and surgery on inflammation-coagulation interaction

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Abstract: Background: The extent of perioperative cross-talk between coagulation and inflammatory markers depends on the type of surgery and anesthesia. **Methods:** After obtaining ethics committee approval and patients' written informed consents, 68 patients aged 25–65 years of either sex, American Society of Anesthesiology (ASA) physical status I–II were randomly assigned to one of four equal groups: Open or Laparoscopic cholecystectomy groups anesthetized with either Isoflurane (IO or IL groups) or Sevoflurane (SO or SL groups) in order to assess coagulation, anticoagulation, fibrinolytic and inflammatory markers preoperatively, immediately postoperative, 24 and 72 hours after surgery. **Results:** In all groups, prothrombin concentration significantly reduced while prothrombin time, partial thromboplastin time and international normalized ratio significantly increased ($P < 0.05$) postoperatively. Coagulation markers including; soluble platelet selectin, von Willebrand factor, thrombin antithrombin complex, D-dimer, as well as inflammatory markers including; high sensitivity C-reactive protein, interleukin- 1 beta and interleukin-6 showed significant ($P < 0.05$) postoperative elevation. Plasminogen and anticoagulation markers including; antithrombin, protein C and S significantly reduced ($P < 0.05$) in all groups. Coagulation and inflammatory markers were significantly higher while plasminogen and anticoagulation markers were significantly lower in open than laparoscopic groups and after isoflurane than sevoflurane anesthesia ($P< 0.05$). Correlations between coagulation and inflammatory markers were observed postoperatively. **Conclusions:** Laparoscopic surgery under sevoflurane anesthesia was associated with less hypercoagulability than open surgery under isoflurane anesthesia probably mediated through inflammatory response.
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1. Introduction

There are increasing evidence points to extensive cross-talk between inflammation and coagulation [1]. Under normal circumstances, the natural anticoagulant mechanisms provide a potent defence against thrombotic complications. Inflammatory mechanisms shift the hemostatic balance to favour the activation of coagulation to the extremes, either disseminated intravascular coagulation or thrombosis. Inflammatory mediators can elevate platelet count, platelet reactivity, down regulate natural anticoagulant mechanisms, initiate the coagulation system, facilitate propagation of the coagulant response and impair fibrinolysis. Whereby inflammation leads not only to activation of coagulation and fibrin deposition, but coagulation also considerably affects inflammatory activity both by releasing mediators from platelets and other activated cells [2].

Surgery is followed by changes in coagulation and fibrinolytic systems with a surge in circulating cytokines, which may favour the development of postoperative thromboembolic complications that can occur after open and laparoscopic surgery [3]. Only a few randomized studies have addressed the issue of comparing the alterations of hemostasis and its relation to inflammatory response after laparoscopic and open surgery using different types of anesthesia.

Anesthestists are often involved in the peri-operative management of hemostasis in order to reduce the incidence of postoperative thrombosis or bleeding. Various studies failed to elucidate the exact effect of inhalation anesthetics such as halothane, isoflurane and sevoflurane on hemostasis. Some *in vitro* studies showed suppression of platelet aggregation [4,5] while another *in vivo* study found no inhibition at all [6]. Moreover, it is possible that some of these anesthetic agents might protect against intravascular thrombosis during surgery [7].

Thus, we hypothesized that the inflammatory response to surgery might have an impact on coagulation under various anesthetic agents. This prospective randomized study was designed to assess the changes and correlations between hemostatic and inflammatory markers in patients undergoing laparoscopic versus open cholecystectomy anesthetized by sevoflurane or isoflurane. This might help us to

know the type of surgical technique and anesthetic drug with the least effect on hemostatic mechanism and inflammatory response.

2. Materials and Methods

After obtaining Institutional Ethics Committee approval and informed written patients' consents, 68 patients aged 25–65 years of either sex, American Society of Anesthesiology (ASA) physical status I–II scheduled for elective cholecystectomy were included in this study. Patients with obesity (body mass index > 35 kg/m^2), chronic liver disease, pregnant or lactating women, malignant, feverish or septic cases or patients with compromised cardiac or pulmonary function were excluded from the study. Patients with history of hemostatic abnormalities, those on medications known to affect hemostatic or immunological response, or who required blood or colloid transfusion were also excluded.

The patients were assigned by computer generated random numbers into 2 main groups (34 patients each): Group (I) received isoflurane anesthesia and group (S) received sevoflurane anesthesia. Each group was further subdivided into two subgroups of 17 patients each: IO and SO subgroups performed open cholecystectomy while IL and SL subgroups performed laparoscopic cholecystectomy.

Premedication consisted of midazolam (0.05 mg/kg), ondansetron (4 mg), metaclopramide (10 mg) and ranitidine (50 mg) was given intravenously half an hour before operation. In the operating room the following monitors were attached to the patients: 5 leads ECG, non-invasive arterial blood pressure, peripheral oxygen saturation, end-tidal carbon dioxide tension, inhaled anesthetic gas analysis, temperature and neuromuscular monitoring (Infinity Kappa, Dräger, Lübeck, Germany). Anesthesia was induced with fentanyl (2–3 µg/kg), propofol (2–2.5 mg/kg) and atracurium (0.5 mg/kg and maintained with 1 MAC sevoflurane (group S) or 1 MAC isoflurane (group I) in 30% O_2 in air using a low flow (2 L/min) circle system. Supplemental doses of fentanyl and atracurium were given when needed. All the patients received crystalloids at a rate of 5–7 ml/kg/h during surgery. No colloid or blood transfusion was allowed. Postoperative analgesia was provided by intravenous infusion of 1 gm acetaminophen and IM mepridine 1 mg/kg every 12 hours.

Hematological studies

Four venous blood samples (10 ml each) were collected; before operation, immediate post-operative, 24 hours and three days after surgery. The following laboratory investigations were performed:

- Complete blood counts including; hemoglobin concentration (Hb), hematocrit (Hct) and platelet count (PLT). All were measured by automatic hematology analyzer Celltac-MEK 8118 (Nihon Kohden, Japan).
- Screening coagulation tests; prothrombin time (PT), prothrombin concentration, international normalized ratio (INR) and partial thromboplastic time (PTT). They were measured by fibrin timer (Behring werke AG, Marburg).
- Specific coagulation parameters [soluble platelet selectin (sP-selectin), fibrinogen (FIB), von Willebrand factor (vWF) and thrombin-antithrombin (TAT) complex], anticoagulants [antithrombin (AT), protein C and protin S total antigen], fibrinolytic protein (plasminogen), simultaneous coagulation and fibrinolytic parameter (D-dimer) and inflammatory markers [high sensitivity C-reactive protein (hs-CRP), interleukin-1 beta (IL-1β), interleukin-6 (IL-6)]. All previously mentioned parameters were measured by colorimetric enzyme-linked immunosorbent assay (ELISA) test kits according to the manufacturers` protocols.
- Preoperative liver function tests (ALT, AST, albumin and total bilirubin) and kidney function test (serum creatinine) were performed by the conventional methods to exclude liver and kidney dysfunction.

Statistical Analysis

Based on previous study [8], sample size was calculated according to the postoperative change of D-dimer in patients anesthetized by isoflurane (G power was 3.1). Assuming that α= 0.05 and 1- β (power level) = 80%, effect size was 0.777 so 17 patients per group were required to detect the difference. Results are expressed as median (minimum-maximum) or number (%). Comparison between the four groups was performed using Kruskal-Wallis test. Comparison relative to baseline within the same group was achieved by using Wilcoxon sign rank test. Comparison between categorical data was done using Chi square test. Spearman correlation was used to do correlation between different parameters. The data were considered significant if P value was ≤ 0.05. Statistical analysis was performed with the aid of the SPSS computer program version 12 windows (IBM, USA).

3. Results

All demographic data, duration of surgery and routine preoperative investigations of liver function tests and serum creatinine were statistically comparable between groups.

Hb and Hct levels showed significant ($P < 0.05$) postoperative decrease in all groups when compared to the preoperative values but without significant differences between groups (Table 1). There was a significant ($P < 0.05$) reduction in postoperative prothrombin concentration level as well as increase (P

< 0.05) in *PT, INR and PTT* at different postoperative times in all groups when compared to the baseline value. There was no marked difference between groups except significant ($P < 0.05$) increase in *INR* in SL group at all postoperative times while *PT* was reduced ($P < 0.05$) in IO group immediate and 24 hours postoperatively when compared to other groups (Table 1). Significant ($P < 0.05$) reduction in *PLT* count was observed at immediate postoperative time followed by elevation ($P < 0.05$) at 72 hours postoperatively when compared to preoperative level in all groups without significant differences between groups (Table 1).

Table 1. Results of Pre- and Post-operative (PO) Hemoglobin, Hematocrit, Platelet Count and Screening Coagulation Tests

		IO (n = 17)	IL (n = 17)	SO (n = 17)	SL (n = 17)
Hb (g/dl)	Pre	13.0 (9.4-15.6)	13.0 (9.7-16.5)	12.6 (11.1-16.2)	13.0 (11.0-17.1)
	PO	11.6 (8.5-13.4) *	11.5 (8.4-15.8) *	11.2 (8.4-13.1) *	11.5 (9.5-15.1) *
	1st PO	12.0 (8.7-13.8) *	11.4 (8.9-14.5) *	11.7 (9.0-13.9) *	11.5 (10.2-15.5) *
	3rd PO	11.8 (9.2-14.4) *	11.4 (8.7-14.5) *	12.1 (9.4-14.8) *	12.0 (9.3-15.2) *
Hct (%)	Pre	39.6 (28.9-45.1)	38.2 (29.3-47.5)	40.1 (32.9-45.9)	39.9 (36.1-49.7)
	PO	33.3 (25.5-40.8) *	34.2 (26.2-43.7) *	30.4 (27.7-35.3) *	34.1 (27.6- 44.7) *
	1st PO	35.3 (25.5-41.5) *	34.0 (27.8-43.1) *	33.8 (30.6-73.4) *	35.2 (30.1-45.3) *
	3rd PO	36.3 (26.8-42.5) *	34.2 (27.2-43.2) *	36.1 (31.0-40.2) *	38.2 (31.0-46.0) *
PT (sec)	Pre	11.5 (10.0-13.2)	12.1 (10.6-13.7)	11.8 (10.8-13.4)	11.5 (10.5-13.1)
	PO	13.5 (11.9-18.0) *	14.8 (11.6-18.1) *†	13.8 (12.7-19.6) *	15.7 (12.4-18.6) *†
	1st PO	12.5 (11.5-16.5) *	13.5 (11.0-16.6) *†	13.2 (11.5-16.6) *†	14.0 (11.6-16.5) *†
	3rd PO	11.7 (11.1-15.2) *	12.6 (10.8-14.5) *	12.5 (10.9-14.3) *	13.0 (11.1-14.3) *
Prothrombin concentration (%)	Pre	90.8 (70.8-118.0)	100.0 (80.8-122.9)	92.4 (78.2-120.0)	98.2 (75.9-111.3)
	PO	76.6 (52.1-90.9) *	76.9 (62.6-91.2) *	75.8 (65.3-86.8) *	76.4 (63.8-95.3) *
	1st PO	78.1 (61.5-95.9) *	89.6 (61.5-105.0) *	80.8 (70.3-98.3) *	88.7 (67.1-98.9) *
	3rd PO	83.3 (66.2-111.3) *	92.0 (79.5-105.0) *	88.0 (73.1-113.5) *	90.7 (70.2-100.2) *
INR	Pre	1.0 (0.9-1.2)	1.0 (0.8-1.2)	1.0 (0.9-1.1)	1.0 (0.9-1.3)
	PO	1.3 (1.1-1.4) *	1.3 (1.0-1.9) *	1.3 (1.1-1.4) *	1.5 (1.2-1.8) *†‡§
	1st PO	1.2 (0.9-1.3) *	1.2 (1.0-1.7) *	1.2 (1.1-1.3) *	1.3 (1.1-1.6) *†‡§
	3rd PO	1.1 (1.0-1.4) *	1.2 (0.9-1.3) *†	1.1 (1.0-1.2) *	1.2 (1.0-1.4) *†§
PTT (sec)	Pre	28.8 (25.7- 37.0)	29.2 (24.3-33.1)	28.8 (25.7-35.1)	28.8 (25.6-36.6)
	PO	37.2 (25.5-49.1) *	37.3 (30.4-43.6) *	38.7 (32.2-45.6) *	39.0 (33.1-49.5) *
	1st PO	35.2 (30.5-42.1) *	34.2 (30.1-37.2) *	35.6 (30.8-40.1) *	35.8 (30.0-42.6) *
	3rd PO	32.0 (28.0-40.3) *	31.4 (28.0-42.8) *	32.2 (27.9-38.8) *	32.4 (28.1-40.7) *
PLT (10^9/L)	Pre	275.0 (130-589)	277.0 (142-371)	210.0 (140-397)	278.0 (130-478)
	PO	218.0 (89-502) *	251.0 (95-339) *	181.0 (110-310) *	220.0 (95-460) *
	1st PO	267.0 (128-478)	246.0 (141-326)	235.0 (188-368)	303.0 (111-480)
	3rd PO	328.0 (170-598) *	311.0 (150-412) *	254.0 (171-464) *	312.0 (140-482) *

Data are expressed as median (minimum- maximum). I; patients anesthetized with isoflurane, S; patients anesthetized with sevoflurane, underwent O; open cholecystectomy or L; laparoscopic cholecystectomy. Hb: hemoglobin, Hct: hematocrit, PT: prothrombin time, INR: international normalized ratio, PTT: partial thromboplastin time, PLT: platelet count. PO; postoperative. *P < 0.05 relative to preoperative within the same group. †P < 0.05 relative to IO group. ‡P < 0.05 relative to IL group. §P < 0.05 relative to SO group.

Coagulation markers (*sP-selectin, vWF, TAT, D-dimer*) were significantly ($P < 0.05$) elevated in all postoperative times versus baseline while fibrinogen level was reduced ($P < 0.05$) by the end of surgery followed by an elevation at 24 and 72 hrs afterwards in all groups. All the above markers were significantly ($P < 0.05$) higher postoperatively in open than laparoscopic groups and after isoflurane than sevoflurane anesthesia. The highest levels were in IO group and the lowest in SL group (Tables 2 and 3). In all groups, *plasminogen, AT, protein C and S* showed significant ($P < 0.05$) postoperative reduction yet still higher in laparoscopic versus open surgery and in sevoflurane versus isoflurane groups (Table 3).

Table 2. Results of Pre- and Post-operative (PO) Specific Coagulation Parameters

		IO (n= 17)	IL (n = 17)	SO (n = 17)	SL (n = 17)
sP-selectin (ng/ml)	Pre	65.1 (40-119)	64.8 (41-117)	57.6 (32-114)	66.6 (34-116)
	PO	200 (101-395) *	100 (68-202) *†	120.0 (80-225) *†	100 (58-170) *†§
	1st PO	270 (150-480) *	145 (80-260) *†	180.0 (95-320) *†‡	110 (70-190) *†§
	3rd PO	180 (95-302) *	120 (60-230) *†	100.0 (80-280) *†	90 (62-168) *†§
vWF (%)	Pre	91.0 (65-140)	85.0 (55.3-113.8)	90.7 (47-142)	83.0 (52-124)
	PO	185.0 (112-292) *	112.2 (78.0-185) *†	123.0 (86.6-260) *†	103.0 (60-149) *†§
	1st PO	240.0 (172-365) *	153.0 (99.5-204) *†	190.5 (100-355) *†	125.0 (75-185) *†‡§
	3rd PO	155.2 (110-202) *	112.0 (68-160) *†	122.0 (77-190) *†	96.4 (62-140) *†§
FIB (mg/ml)	Pre	3.7 (1.8-4.5)	3.5 (2.5-4.9)	2.9 (1.6-4.9)	3.1 (1.5-4.8)
	PO	2.6 (1.8-3.6) *	1.9 (1.0-2.4) *†	2.4 (1.2-3.8) *‡	1.9 (1.1-3.0) *†§
	1st PO	7.5 (3.7-8.8) *	5.7 (3.8-7.5) *†	5.1 (2.5-7.8) *†‡	3.9 (2.5-6.2) *†§
	3rd PO	11.1 (6.2-14.4) *	8.9 (6.1-12.0) *	8.0 (4.0-12.5) *†	7.6 (4.5-11.6) *†‡
TAT (ng/ml)	Pre	1.6 (0.8-2.7)	1.8 (0.5-2.9)	1.7 (0.7-2.5)	1.8 (0.5-2.9)
	PO	4.5 (2.6-7.3) *	3.2 (0.9-5.9) *†	3.8 (1.4-6.0) *†	2.7 (0.8-4.0) *†§
	1st PO	6.5 (3.5-10.8) *	4.0 (1.2-6.4) *†	5.1 (2.1-7.5) *†	3.0 (1.0-4.8) *†‡§
	3rd PO	5.2 (2.9-8.4) *	3.0 (0.9-4.5) *†	3.9 (1.8-5.0) *†	2.0 (1.0-4.5) *†§

Data are expressed as median (minimum- maximum). I; patients anesthetized with isoflurane, S; patients anesthetized with sevoflurane, underwent O; open cholecystectomy or L; laparoscopic cholecystectomy. PO; postoperative. sP-selectin: soluble platelet selectin, vWF: von Willebrand factor, FIB: fibrinogen, TAT: thrombin antithrombin complex. *P < 0.05 relative to preoperative (baseline) within the same group. †P < 0.05 relative to IO group. ‡P < 0.05 relative to IL group. §P < 0.05 relative to SO group.

Table 3. Results of Pre- and Post-operative (PO) Anticoagulation and Fibrinolytic Parameters

		IO (n = 17)	IL (n = 17)	SO (n = 17)	SL (n = 17)
AT (µg/mL)	Pre	310.0 (180-400)	270.0 (150-402)	260.0 (140-360)	290.0 (170-440)
	PO	210.0 (140-320) *	230.0 (112-380) *	215.0 (100-301) *	260.0 (143-382) *§
	1st PO	160.0 (102-220) *	185.0 (98-300) *	169.0 (90-230) *	217.0 (127-330) *†§
	3rd PO	220.0 (122-312) *	225.0 (122-335) *	229.0 (108-318) *	240.0 (132-365) *
Protein C (%)	Pre	93.2 (75.3-142.6)	96.6 (76.5-142.6)	89.5 (75.3-142.6)	95.7 (73.0-142.6)
	PO	75.6 (58.8-103.1) *	90.3 (68.9-122.6) *†	79.3 (60-110.4) *‡	93.7 (70.0-121.8) *§
	1st PO	50.5 (42.8-75.4) *	70.3 (55.6-101.1) *†	52.6 (45-90.1) *‡	72.5 (54.8-100.1) *†§
	3rd PO	77.8 (53.0-101) *	80.4 (60.3-121.3) *	78.7 (62-121.3) *	82.7 (65.2-121.3) *
Protein S (%)	Pre	81.8 (70.3-125.4)	75.4 (62.4-110)	81.8 (66.8-96.4)	81.1 (65.6-125.4)
	PO	65.3 (59.4-98.9) *	69.3 (52.9-90.9) *	70.4 (55.4-82.4) *	72.4 (41.6-110.9) *
	1st PO	43.8 (38.2-65.2) *	53.6 (45.2-76.3) *†	51.1 (40.6-60.6) *†	55.7 (44.7-96.7) *†
	3rd PO	68.3 (50.4-95.4) *	69.3 (52.4-96.6) *	69.4 (60.4-89.9) *	70.3 (53.9-120.6) *
Plasmino-gen (µg/mL)	Pre	210.0 (155-295)	200.0 (150-310)	210.0 (150-300)	212.0 (150-300)
	PO	159.0 (123-230) *	185.0 (132-265) *†	187.0 (142-245) *†	210.0 (152-261) †§
	1st PO	109.0 (95-155) *	150.0 (102-220) *†	140.0 (104-197) *†	165.0 (117-223) *†§
	3rd PO	162.0 (112-252) *	177.0 (110-282) *	188.0 (130-260) *	191.0 (128-280) *†
D-dimer (ng/mL)	Pre	280.0 (200-370)	280.0 (112-350)	310.0 (200-400)	250.0 (180-380)
	PO	550.0 (350-790) *	380.0 (180-550) *†	450.0 (290-720) *	334.0 (250-500) *†§
	1st PO	715.0 (410-900) *	480.0 (213-630) *†	550.0 (380-840) *†‡	410.0 (325-580) *†§
	3rd PO	520.0 (310-780) *	400.0 (200-580) *†	430.0 (250-620) *	380.0 (280-486) *†§

Data are expressed as median (minimum- maximum). I; patients anesthetized with isoflurane, S; patients anesthetized with sevoflurane, underwent O; open cholecystectomy or L; laparoscopic cholecystectomy. PO; postoperative. AT: antithrombin. *P < 0.05 relative to preoperative (baseline) within the same group. †P < 0.05 relative to IO group. ‡P < 0.05 relative to IL group. §P < 0.05 relative to SO group.

The inflammatory markers (hs-CRP, IL-1β and IL-6) showed significant (P < 0.05) postoperative elevation in all groups being more marked in patients undergoing open surgery compared to laparoscopic procedure. Sevoflurane anesthesia was associated with lower levels of IL- 6 and IL-1β compared to isoflurane anesthesia. The highest levels were observed in the IO group while the lowest were in SL group (Table 4).

Table 4. Results of Pre- and Post-operative (PO) Inflammatory Markers

		IO (n = 17)	IL (n = 17)	SO (n = 17)	SL (n = 17)
hs CRP (mg/L)	Pre	4.8 (0.9-7.0)	4.8 (1.6-8.9)	4.0 (1.5-6.9)	3.9 (1.6-5.9)
	PO	14.2 (4.0-19.9) *	8.3 (1.9-16.3) *†	10.1 (2.1-17.8) *†	7.3 (4.0-12.0) *†§
	1st PO	18.8 (6.1-28.6) *	12.4 (4.0-24.5) *†	14.2 (4.0-21.0) *†	11.2 (5.0-18.6) *†§
	3rd PO	27.5 (8.4-36.2) *	16.8 (5.6-31.2) *†	21.5 (6.5-30.4) *†	15.6 (6.5-23.6) *†§
IL-1β (pg/ml)	Pre	3.0 (1.5-5.1)	3.5 (2.1-5.2)	2.8 (1.5-4.6)	3.2 (1.6-4.3)
	PO	10.8 (5.3-17.8) *	8.8 (4.1-12.1) *†	8.5 (4.4-13.8) *†	6.3 (3.3-8.6) *†‡§
	1st PO	13.5 (7.3-23.0) *	11.2 (5.7-15.6) *†	11.2 (5.3-16.8) *†	9.4 (4.6-12.8) *†‡§
	3rd PO	17.1 (8.6-28.2) *	14.1 (7.5-20.8) *†	13.4 (7.2-22.1) *†	11.5 (6.0-15.1) *†‡§
IL-6 (pg/mL)	Pre	2.8 (1.0-4.5)	2.8 (0.9-5.0)	2.4 (0.9-4.0)	2.8 (0.7-5.1)
	PO	15.6 (10-20.8) *	10.9 (5.6-17.5) *†	12.1 (6.8-15.9) *†	8.4 (2.1-15.3) *†‡§
	1st PO	18.6 (11.6-23.4) *	12 (6.5-20.1) *†	12.6 (6.3-18.1) *†	9.8 (3.4-16.1) *†§
	3rd PO	19.5 (10.1-27.2) *	14 (7.2-22.5) *†	16.1 (9.8-20.8) *†	11.2 (2.7-20.4) *†§

Data are expressed as median (minimum- maximum). I; patients anesthetized with isoflurane, S; patients anesthetized with sevoflurane, underwent O; open cholecystectomy or L; laparoscopic cholecystectomy. PO; postoperative. hs CRP: high sensitivity C-reactive protein, IL-1β: interleukin 1 beta, IL-6: interleukin-6. *$P < 0.05$ relative to preoperative (baseline) within the same group. †$P < 0.05$ relative to IO group. ‡$P < 0.05$ relative to IL group. §$P < 0.05$ relative to SO group.

Statistical correlations were performed between sP-selctin (as a marker of platelet activation), AT (as a marker of anticoagulant activity) and D-dimer (as a marker of simultaneous activation of coagulation and fibrinolysis) versus the three inflammatory markers (IL-1β, IL-6 and hs CRP). There were significant positive correlations between IL-6 and D-dimer in IO group immediately postoperative (r = 0.525, $P = 0.031$) and after 24 hours (r = 0.647, $P = 0.005$), as well as between IL-1β and D-dimer in SO group (r = 0.656, P value = 0.004) and (r = 0.552, $P = 0.021$) at the same times respectively. In IL group, the correlation was significantly negative between IL-6 and AT (r = -0.497, $P = 0.043$) and significantly positive (r = 0.561, P = 0.019) between sP-selectin and hs-CRP 24 hours postoperatively.

4. Discussion

This study revealed that open surgery under isoflurane anesthesia were associated with higher coagulation and inflammatory markers with less anticoagulation factors than laparoscopic surgery under sevoflurane anesthesia.

Results of the present study revealed significant reduction in PLT count in the 1st post operative day compared to preoperative levels in all groups which can be explained by the hemodilution occurred following surgery [9]. Similar results were found by Diamantis *et al.* [10] and Brueckner *et al.* [8]. Then PLT count was significantly increased on the 3rd postoperative day with postoperative increase of platelet reactivity in the form of higher levels of both sP-selectin and vWF compared to baseline values. The highest levels of both elements were observed in the IO group and the lowest levels were in the SL group. These results may be explained by the increased inflammatory response noticed in the patients undergoing open surgery [11] with more liability to thrombin formation [12] as higher platelet responsiveness lead to release of ultra-large von Willebrand factor multimers from the endothelium [13]. Then vicious circle occurs as activated platelets are capable of releasing high concentrations of the pro-inflammatory mediator, CD40 ligand which increases inflammatory cytokines, such as IL-6 and IL-8 [14]. In addition, sevoflurane anesthesia has been shown to have a suppressive effect on platelet aggregation which is not the case with isoflurane during *in-vitro* and *in-vivo* studies [4,6].

Significant increase in PT, INR and PTT in the 1st post operative day was detected compared to preoperative levels. This may be explained by the hemodilution following surgery since they returned to almost baseline on third postoperative day [9]. Significant PT decrease in IO group was noticed when compared to other groups which is also observed in other studies during open surgery [15] and under isoflurane anesthesia [8,16]. On the other hand Sun [17] found that there was no significant difference in screening coagulation parameters such (PT, PTT and TT) or thromboelastographic coagulation variables between sevoflurane and isoflurane anesthesia.

TAT forms when AT binds with thrombin thus it is considered an index of clot formation [18]. TAT level in the current study increased significantly at the end of the operation in the four groups and continued till the third postoperative day showing that thrombin generation and hypercoagulability started immediately after surgery and continued afterwards. TAT level was higher in the open surgery groups than laparoscopic groups in both types of anesthesia and were lower in sevoflurane groups when compared to isoflurane groups in both types of surgery. These results go in accordance with Diamantis *et al.* [10]. Also Nguyen *et al.* [18] found a significant increase of TAT level after open or laparoscopic gastric by-pass (GBP) over baseline levels but without significant differences between groups. The discrepancy in results when comparing both groups could be attributed to the chosen model of operation (GBP) which is associated with more surgical stress.

In the present study, there was a significant reduction of fibrinogen level at the end of surgery (due to its consumption and/or hemodilution). Then it increased and reached maximum levels at 72 h postoperatively in all groups noted mainly in the IO group. Fibrinogen is known to behave as an acute phase reactant with stores released at time of physiological stress [19]. Similar results were given by Diamantis *et al.* [10] and Nguyen *et al.* [18] but without significant difference between open and laparoscopic surgery in the later study. Sun [17] found no significant postoperative difference in fibrinogen level in patients who underwent breast cancer surgery whether anesthetized by sevoflurane or isoflurane or between groups but take in consideration that coagulation profile may be disturbed in cancer patients.

Plasminogen is the precursor to plasmin which is, in turn, the primary molecule for fibrin degradation. Our results revealed significant postoperative reduction in all groups which extended up to the third postoperative day. The increase in fibrinogen with a concomitant decrease in plasminogen may also favor increased fibrin formation. Our results were in accordance with Nguyen *et al.* [18].

D-dimer is frequently increased after surgery or trauma and indicates the presence of intravascular clot that has undergone lysis [20]. D-dimer level increased postoperatively in all groups but significantly higher in the open cholecystectomy groups than in the laparoscopic cholecystectomy groups in both types of anesthesia. This indicates simultaneous activation of blood coagulation and fibrinolysis which is more enhanced in the open surgery. The levels of D-dimer were lower in sevoflurane group than isoflurane groups in both types of surgery. The higher postoperative increase in D-dimer level was also obtained by Diamantis *et al.* [10] and Khafagy *et al.* [16].

The present study revealed a significant postoperative reduction of AT, protein C and protein S in all groups. These antithrombotic parameters were higher in SL group. Similarly, Nguyen *et al.* [18] found postoperative reduction of AT and protein C in the open and laparoscopic GBP surgery but was significantly higher in the open group. The same results were obtained by Papaziogas *et al.* [11] concerning AT level and by Schietroma *et al.* [3] concerning AT and protein C levels but without significant difference between open and laparoscopic cholecystectomy.

The conflicting results of the hemostatic parameters in different studies may be related to the different sensitivity of test kits used, duration and type of surgical procedure, type of anesthetic drugs and techniques, and whether the study was in-vitro or in-vivo. This requires careful interpretation of results according to the surrounding circumstances. Also, the impact of stress response and the role of inflammatory markers effect on the process of blood coagulation and fibrinolysis should be taken in consideration.

As regards the inflammatory markers hs CRP, IL-1β and IL-6, the current study showed significant postoperative elevation in all groups which was more marked in patients undergoing open surgery compared to laparoscopic procedure. This may be explained by greater tissue damage inflicted by the open technique. CRP is an acute phase protein whose levels are roughly proportional to the magnitude of the trauma [3]. IL-6 induces hepatic synthesis of CRP and may be a faster reacting and more accurate gauge of tissue damage than CRP [21]. Rahr *et al.* [22] reported similar results in open and laparoscopic recurrent inguinal hernia but without significant difference between the two groups. The present study also showed that sevoflurane anesthesia was associated with lower levels of IL-6 and IL-1β than isoflurane anesthesia. The highest levels of IL-6 and IL-1β were observed in the IO group and the lowest levels were in SL group. Moreover, significant positive correlations were noticed between both IL-6 and IL-1β and D-dimer in IO group. This correlation supports the observation that inflammation stimulates coagulation, resulting in hypercoagulable state that should be considered during postoperative patient follow up [23, 10].

In conclusion, laproscopic surgery under sevoflurane anesthesia showed less activation of coagulation and inflammatory response than open surgery under isoflurane anesthesia. The cytokine surge was correlated to enhanced coagulation which might be related to the type of surgery performed and the type of anesthesia used. Thus, laparoscopic surgery under sevoflurane anesthesia is recommended for high risk patients of thromboembolic complications as ischemic heart disease. We also recommend new studies to understand the mechanisms involved in the crosstalk

between inflammation and coagulation as this may yield new therapeutic strategies for human diseases and to be translated into differences in clinical outcome.

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