COAGULATION AND FIBRINOLYTIC CHANGES AFTER LAPAROSCOPIC CHOLECYSTECTOMY: A PROSPECTIVE CLINICAL STUDY

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ABSTRACT

Objective: Laparoscopic surgery appears to be less traumatic to the patient than open surgery, but its influence upon haemostatic parameters is incompletely elucidated. The aim of this study was to investigate changes in coagulation and fibrinolysis following laparoscopic cholecystectomy (LC) as compared to open cholecystectomy (OC), to determine whether changes occur after LC that may indicate a risk of thrombosis.

Patients and Methods: Forty-one patients who underwent either LC (22 patients, study group) or OC (19 patients, control group) for uncomplicated cholelithiasis were prospectively enrolled. Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, antithrombin III (AT III), thrombin-antithrombin complexes (TAT), fibrin monomers (FM), prothrombin fragment 1+2 (F1+2) and d-dimer were measured before and at 6, 12, and 24 hrs after operation in all patients.

Results: No statistical difference was found comparing pre- and post-operative values of PT, aPTT or AT III at all determinations in either group or in-between the two groups. There were significant increases of serum concentrations of fibrinogen, TAT, FM, F1+2, and D-dimer in all post-operative determinations as compared to pre-operative values in LC and OC with no significant differences between the 2 groups.

Conclusion: The data of this study suggest that LC induces activation of coagulation and fibrinolytic pathways similar to those following OC. Laparoscopic cholecystectomized patients should be considered at risk for thromboembolism. Therefore, it is recommended that deep vein thrombosis prophylaxis should be utilized in laparoscopic as in conventional open cholecystectomy patients.

INTRODUCTION

Activation of coagulation and fibrinolysis occurs as a stress response to surgery and may predispose the patient to thrombo-embolic complications (1,2). Some investigators have suggested that laparoscopic cholecystectomy (LC) may lead to a higher risk of postoperative thrombo-embolic events than conventional open cholecystectomy (OC) (3,4). At present the incidence of deep vein thrombosis (DVT) and pulmonary embolism (PE) after laparoscopy is not known. There are reports with contradictory results (4,5). In addition, there are no conclusive biological studies to establish the existence of haemostatic alterations that would indicate a risk of thrombosis following laparoscopic cholecystectomy (6-9).

The aim of this study is to investigate changes in coagulation and fibrinolysis following LC compared to OC and to determine whether changes occur after LC that may indicate a greater risk of thrombosis.

PATIENTS AND METHODS

The study was approved by the local ethics committee of Dallah hospital, Riyadh, KSA and informed consent was obtained from participating patients. Forty-one adult patients classified as American Society of Anaesthesiologist physical status I or II who underwent either LC (22 patients, study group) or OC (19 patients, control group) for uncomplicated cholelithiasis were prospectively enrolled.

Patients were excluded from the study if they had thrombo-embolic disease or a previous history of it, coagulation disturbances, a recent transfusion of blood or one of its products, evidence of neoplasia and those on anticoagulant therapy or anti-platelet agents.

Anaesthetic technique was standardized for all patients. They were premedicated with lorazepam 2 mg orally 2 hrs before operation and anaesthesia was induced with fentanyl 2 ug.Kg⁻¹ and propofol 2 mg.Kg⁻¹. Atracurium 0.5 mg.Kg⁻¹ was administered to facilitate endotracheal intubation. Anaesthesia was maintained with 1-1.5% sevoflurane and 60% nitrous oxide in oxygen. Neuro-muscular block was maintained by continuous infusion of atracurium to elicit one response of train of four throughout the procedure, as determined by the neurostimulator (Myotest, Biometer International Ltd, Odense, Denmark). The ventilator settings (North American Drager, Narkomed 2C, USA) were adjusted to maintain end-tidal CO₂ tension at 35-40 mmHg. Antibiotic prophylaxis was achieved by third-generation cephalosporin. Fluid balance was maintained by Ringer’s lactate infused at a rate of 10-15 ml.Kg⁻¹.h⁻¹. At the end of surgery residual neuromuscular blockade was antagonized with neostigmine 40 ug.Kg⁻¹ and atropine 15 ug.Kg⁻¹ intravenously. Ambulation was started on first and third postoperative days in LC and OC groups respectively. Patients were discharged from the hospital on the second and fifth postoperative days in LC and OC groups respectively.
Laparoscopic cholecystectomies were performed with the patient in 30 degrees reverse Trendelenburg position, using four-trocar technique<sup>33</sup>. The abdomen was insufflated with CO<sub>2</sub> via an automatic insufflator set at 1L.min<sup>-1</sup> and the intra-abdominal pressure was maintained at 12 mmHg. Open cholecystectomies were performed through a transverse right subcostal incision, 10-12 cm long, with partial transection of the ipsilateral rectus abdominis muscle. Mechanical prophylaxis against DVT in the form of elastic compression stockings was used in all patients. No pharmacological prophylaxis was used in order to avoid their possible effects on measured coagulation parameters. A venous duplex scan of both legs was performed on 7<sup>th</sup> postoperative day, at the time of patients` routine follow up visit in outpatient surgery clinic.

**Haemostatic assay:**

Venous blood samples for determination of haemostatic parameters were obtained from all patients at the following times: - (a) under basal conditions in the morning on the day of the operation, T0; (b) 6 hours after surgery, T1; (C) 12 hours after surgery, T2; and (D) 24 hours after surgery, T3.

Polyethylene tubes (2 X 5 ml) containing 1/10 volume of 0.129 M trisodium citrate (Becton Dickinson, New Jersey, USA) were used to collect the blood samples. Platelet poor plasma was obtained by centrifugation at 3000 g for 10 min at –70 degree centigrade. These samples were thawed immediately before the assay procedure.

The following haemostatic parameters were examined: prothrombin time (PT) and activated partial thromboplastin time (aPPT) were studied with thromboplastin and activated cephaloplatin, respectively (IL, Dade, Barcelona, Spain). These coagulation assays were carried out with an ACL 6000 coagulometer System, Milano, Italy. The reference ranges for PT and aPPT were 11.4-14 sec and 25.0-39.0 sec respectively. Thrombin-antithrombin complexes (TAT) and prothrombin fragment (F1+2) were analyzed using an ELISA kit (Behringwerke AG, Marburg, Germany). Reference intervals for TAT and F1+2 were 1.2 - 5.0 ug.L<sup>-1</sup> and 0.4 - 1.5 nmol.L<sup>-1</sup> respectively. Fibrinogen was measured by prothrombin time derivate assay (IL, Dade, Barcelona, Spain) with a reference range of 178-450 mg% (i.e. per 100 ml). Fibrin monomers (FM) were measured using a spectrophotometric assay (Behricham, Behringwerke AG, Marburg, Germany). The reference interval was 2.8-17.3 mg.L<sup>-1</sup> with a median value of 9 mg.L<sup>-1</sup>. Human cross-linked fibrin degradation products (D-dimer, D-D), as a marker of plasmin activity, were determined by an enzyme immunoassay (Enzygrost D-dimer micro, Behring Diagnostics Gmbh, Marburg, Germany), with a reference interval of 4-78 µg.L<sup>-1</sup>. The plasmatic inhibitor antithrombin III (AT III) activity was determined by chromogenic substrates using ACL-Future System (IL Coagulation System, Milano, Italy). The reference interval was 65-130%.

Unless otherwise specified, data are presented as mean ± SD. Statistical analysis was performed using SPSS for windows V10.0 (SPSS, Chicago, IL, USA). Data were analyzed with Mann Whitney test with Bonferroni correction for multiple comparisons, Wilcoxon signed rank test and student`s paired and un-paired t-test. A P value < 0.05 was considered statistically significant.

**RESULTS**

Patients` demographics did not differ between the two groups. Duration of anaesthesia and surgery did not differ between laparoscopic and open cholecystectomy groups (table 1). All haemostatic variables reflected values within a normal range in basal pre-operative samples with no significant differences between the 2 groups (table 2).

Coagulation and fibrinolytic data for each parameter at specified time of measurement are shown in figures 1-8. No statistical difference was found comparing pre- to post-operative values of PT, aPPT or AT III at all determinations in either group or in between the two groups (figures 1,2,4).

Conversely, there was a significant increase of serum concentration of fibrinogen, TAT, FM, F1+2 and D-dimer in all post-operative measurements as compared to pre-operative values in LC and OC groups. Post-operative determinations of these parameters were not significantly different between the 2 groups in all samples (figures 3,5,6,7,8).

The time course of serum fibrinogen concentrations is shown in figure 3. In LC group, fibrinogen increased from baseline value of 397 ± 214 mg% to 580 ± 251 at 12 hrs post-operatively, and from 416 ± 218 mg% to 591 ± 253 mg% 6 hrs after operation in OC group. Serum TAT Concentration increased from pre-operative value of 3.9 ± 1.2 ug.L<sup>-1</sup> to 9.1 ± 2.3 ug.L<sup>-1</sup> at 6 hrs post-operatively in LC group and from 3.7 ± 1.3 ug.L<sup>-1</sup> to 9 ± 2.2 ug.L<sup>-1</sup> at 12 hrs post-operatively in OC group (figure 5). The time course of serum FM concentration is shown in figure 6. Peak serum concentrations of FM were achieved at 6 hrs after surgery in both LC and OC groups (48.32 ± 9.62 mg.L<sup>-1</sup> and 52.27 ± 8.36 mg.L<sup>-1</sup> respectively). Serum FM concentration started to decline gradually at 12 and 24 hrs post-operatively in both groups, but remained significantly elevated as compared to pre-operative values. The peak levels of serum F1+2 recorded at 12 hrs after operation (9.32 ± 214 mg%).
112.82 ± 18.36 ug.L-1 at 12 hrs post-operatively in LC group and from 19.82 ± 3.91 ug.L-1 pre-operatively to 109.72 ± 16.31 ug.L-1 at 12 hrs following surgery in OC group (figure 8).

There were no intra- or post-operative complications. None of the patients developed clinical or ultra-sonographic evidence of DVT during their routine follow up visit in outpatient surgery clinic on the 7th post-operative day.

**DISCUSSION**

The incidence of thrombo-embolic events following laparoscopic surgery is reported to be quite low, ranging between 0 and 0.68% (10-16). Only two studies, however, have used objective methods for the surveillance of DVT after laparoscopic cholecystectomy. The first study (6) reported only one DVT in 100 patients (1%) screened by Doppler ultra-sound on the 7th post-operative day. The second study (4) showed DVT in 55% of patients (11 of 19) studied also by Doppler ultra-sound on day 1 after operation. In the present study, none of the patients scanned by Duplex ultra-sound on the 7th post-operative day showed signs suggestive of thrombo-embolic complication. This discrepancy may be explained by the low sensitivity of ultrasonography for the surveillance of DVT in asymptomatic post-operative patients. In addition, Lindberg et al (3) hypothesized that DVT do actually ...

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**Table (1):** Patients' characteristics and operative data in laparoscopic and open cholecystectomy groups.

<table>
<thead>
<tr>
<th></th>
<th>LC Group</th>
<th>OC Group</th>
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<tbody>
<tr>
<td>Number of patients</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32 ± 9</td>
<td>31 ± 11</td>
</tr>
<tr>
<td>Female/Male (%)</td>
<td>17/5 (77.3%)</td>
<td>14/5 (73.7%)</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>72.4 ± 13.6</td>
<td>69.8 ± 11.9</td>
</tr>
<tr>
<td>Duration of anaesthesia (minutes)</td>
<td>116.3 ± 21.4</td>
<td>123.1 ± 23.6</td>
</tr>
<tr>
<td>Duration of operation (minutes)</td>
<td>97.2 ± 17.6</td>
<td>94.6 ± 16.9</td>
</tr>
</tbody>
</table>

LC= laparoscopic cholecystectomy, OC= open cholecystectomy

**Table (2):** Pre-operative values of measured coagulation parameters in laparoscopic and open cholecystectomy groups.

<table>
<thead>
<tr>
<th></th>
<th>LC Group</th>
<th>OC Group</th>
</tr>
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<tbody>
<tr>
<td>PT (sec)</td>
<td>11.9 ± 1.7</td>
<td>12.3 ± 1.6</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>32.5 ± 7.4</td>
<td>33.7 ± 6.2</td>
</tr>
<tr>
<td>Fibrinogen (mg%)</td>
<td>397 ± 214</td>
<td>416 ± 218</td>
</tr>
<tr>
<td>AT III (%)</td>
<td>108 ± 7.9</td>
<td>106 ± 7.4</td>
</tr>
<tr>
<td>TAT (ug.L-1)</td>
<td>3.9 ± 1.2</td>
<td>3.7 ± 1.3</td>
</tr>
<tr>
<td>FM (MG.L-1)</td>
<td>25.16 ± 5.36</td>
<td>26.48 ± 5.41</td>
</tr>
<tr>
<td>F1+2 (nmol.L-1)</td>
<td>2.4 ± 0.31</td>
<td>2.59 ± 0.46</td>
</tr>
<tr>
<td>D-dimer (ug.L-1)</td>
<td>21.16 ± 4.37</td>
<td>19.82 ± 3.91</td>
</tr>
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</table>

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*Figure 1:* Prothrombin time (seconds) at different times. LC = laparoscopic cholecystectomy, OC= open cholecystectomy, T0= pre-operative, T1= 6 hrs postoperatively, T2= 12 hrs postoperatively, T3= 24 hrs postoperatively.
Figure 2: Activated partial thromboplastin time (aPTT, seconds) at different times. LC = laparoscopic cholecystectomy, OC = open cholecystectomy, T0 = pre-operative, T1 = 6 hrs postoperatively, T2 = 12 hrs postoperatively, T3 = 24 hrs postoperatively.

Figure 3: Serum fibrinogen level (mg%) at different times. LC & OC = laparoscopic and open cholecystectomy. T0, T1, T2, T3 = pre-operative, 6, 12, 24 hrs postoperatively. 

* ** = significantly different from preoperative values in LC & OC respectively.

Figure 4: Antithrombin III activity (%) at different times. LC & OC = laparoscopic and open cholecystectomy. T0, T1, T2, T3 = pre-operative, 6, 12, 24 hrs postoperatively respectively.
Figure 5: Serum thrombin antithrombin level (TAT). LC & OC = laparoscopic and open cholecystectomy. T0, T1, T2, T3 = pre-operative, 6, 12, 24 hrs postoperatively. *, ** = significantly different from preoperative values in LC & OC respectively.

Figure 6: Serum fibrin monomers (FM). LC & OC = laparoscopic and open cholecystectomy. T0, T1, T2, T3 = pre-operative, 6, 12, 24 hrs postoperatively. *, ** = significantly different from preoperative values in LC & OC respectively.

Figure 7: Serum prothrombin fragment 1+2 (F1+2). LC & OC = laparoscopic and open cholecystectomy. T0, T1, T2, T3 = pre-operative, 6, 12, 24 hrs postoperatively. *, ** = significantly different from preoperative values in LC & OC respectively.
Figure 8: Serum D-dimer level. LC & OC = laparoscopic and open cholecystectomy. T0, T1, T2, T3= pre-operative, 6,12, 24 hrs postoperatively. *, ** = significantly different from preoperative values in LC & OC respectively.

occur in the immediate post-operative period following LC, but early ambulation prevents thrombi from increasing in size and propagating proximally. This may explain the 55% rate of DVT demonstrated on the first PO day by Patel et al (4) and the 0% and 1% rates found in this study and Caprini et al (6) report, respectively, on day 7 after operation.

Only few investigators have addressed the peri-operative changes of haemostatic parameters following laparoscopic surgery. Lauro et al (17) compared haemostatic changes in OC and LC and recorded significantly higher serum fibrinogen and D-dimer levels in the open group. They concluded that coagulative-fibrinolytic changes are less marked in laparoscopic than in open cholecystectomies. Martinez-Ramos et al (18) examined the fibrinolytic activity in 20 patients following LC. They reported a significant increase in fibrinolysis which, according to the authors, may reduce the thrombo-embolic risk in laparoscopic surgery. Dexter et al (7) compared OC and LC and found similar changes in coagulation and fibrinolysis, except for a longer euglobin clot lysis time and a higher plasminogen activator inhibitor-1 in the open group. Lindberg et al (3) demonstrated significant increases in serum levels of TAT, F1+2 and D-dimer following LC in 64 patients. Similarly, Rahr et al (19) reported a significant rise of serum concentrations of F1+2, soluble fibrin and fibrin degradation products following LC. Caprini et al (20) demonstrated a significant hypercoagulable state in laparoscopic cholecystectomized patients, as shown by thromboelastogram index and aPPT.

The coagulation pathway in this study was assessed by PT, aPTT, fibrinogen, AT III, TAT, FM and F1+2 and they remained significantly elevated for 24 hrs post-operatively. The increase of these coagulation parameters reflects the increase of the corresponding coagulating plasma proteins in the circulating blood. This increase is produced, basically, by a change in the endocrine-metabolic response to trauma resulting from surgery itself which depends, among other factors, on the type and degree of tissue injury induced (2,20). As tissue trauma associated with LC is less than that with OC, a different haemostatic response after laparoscopic surgery when compared to open surgery was anticipated. However, this study, as well as two others (17, 21), was not able to demonstrate a statistical difference between laparoscopic and open cholecystectomies regarding post-operative haemostatic alterations.

Perhaps the greatest difference between LC and OC is the degree of trauma inflicted on the abdominal wall. Trauma to the intra-abdominal organs during LC, however, may be as great as that associated with OC, since the gall bladder is dissected from its bed and the cystic duct and artery are divided just as in OC (7). The dissection in LC is commonly performed with a dissection hook using a bipolar diathermy, which could cause more injury to the hepatocytes than the scissors used during OC (11). Thus it seems possible that the intra-abdominal trauma is even greater following LC (10). In addition, laparoscopic surgery may potentially predispose to thrombosis since it alters venous flow and may cause endothelial injuries (22). Pneumoperitoneum and reverse Trendlenburg position may impair flow in the lower extremities and therefore may induce venous stasis (22-24). Pneumoperitoneum may also damage the vessel wall, causing exposure of the subendothelium with platelet activation (19).
This study was not able to demonstrate changes of other coagulation parameters such as PT, aPTT and AT III. Probably these parameters could have increased earlier after surgery and returned to normal at 6 hrs post-operatively, as was previously shown by other investigators(3, 7, 9).

The results of fibrinolytic assay in the present investigation (increased plasmatic D-dimer) show an enhancement of fibrinolysis following LC. Laparoscopic surgery may induce release of endothelial tissue plasminogen activator through anoxia and injury to the endothelial venous cells (25). The intra-abdominal pressure of pneumoperitoneum at 12 mmHg could provoke this condition on the abdominal venous wall during the 86.3 min, the mean duration of pneumoperitoneum in the present study, which is greater than the 10 min period needed in venous occlusion tests used to determine the individual fibrinolytic activity of the endothelium(22).

In conclusion, the data of this study suggest that LC induces activation of coagulation and fibrinolytic pathways similar to those following OC. Early ambulation after laparoscopic surgery may reduce the incidence of clinically manifest DVT. Nevertheless, laparoscopic cholecystectomized patients may be considered at risk for post-surgical thrombo-embolic complications. Therefore, it is recommended that DVT prophylaxis should be utilized in patients undergoing laparoscopic as in conventional open cholecystectomies.

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REFERENCES


