

Proinflammatory cytokines in open versus laparoscopic cholecystectomy

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ABSTRACT

Objective: Laparoscopic cholecystectomy, a minimal access surgery, is fast replacing open cholecystectomy and is being associated with less trauma. The objective of this study was to compare the proinflammatory cytokine levels in both laparoscopic cholecystectomy and open cholecystectomy.

Methods: This study was carried out at Aseer Central Hospital, Aseer region, Abha Private Hospital and the College of Medicine and Medical Sciences, King Khalid University, Abha, Kingdom of Saudi Arabia, during the time period October 1998 through to November 2000. Sixty-one patients were included in the study, 27 of them had laparoscopic cholecystectomy and 34 had open cholecystectomy. Cytokines [Interleukin-6, Interleukin-1 β , Tumor necrosis factor - α and Interleukin-8] were measured in blood samples collected from the patients before, at and 24 hours post surgery, using commercially available kits.

Results: Interleukin-6 levels were significantly increased at 24 hours post surgery in the open cholecystectomy group of patients compared to the laparoscopic cholecystectomy group ($P < 0.04$). No differences were found in the other cytokines levels (Interleukin-1 β , tumor necrosis factor - α and Interleukin-8) between the open cholecystectomy and laparoscopic cholecystectomy groups.

Conclusion: Laparoscopic cholecystectomy, a minimal access surgery, is associated with lower levels of the proinflammatory interleukin-6 cytokine compared to open cholecystectomy.

Keywords: Cytokines, laparoscopy, cholecystectomy, inflammation.

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Minimal access surgery is designed to reduce the trauma of access inherent in open abdominal surgery. Laparoscopic cholecystectomy (LC), a minimal access surgery, is significantly associated with less postoperative discomfort, and secondary paralytic ileus, better ventilatory function, absence of wound complications, and accelerated recovery, with an early return to full activity compared to open cholecystectomy (OC).¹⁻³ Laparoscopic cholecystectomy, being less invasive, is therefore associated with less trauma and acute inflammation. Proinflammatory cytokines and adhesion molecules

play a major role in acute inflammation following surgical trauma.⁴ Hence, the measurement of these proinflammatory mediators could be used to assess the level of surgical trauma. We measured and compared the level of the proinflammatory cytokines Interleukin (IL)-6, IL-1 β , IL-8 and tumor necrosis factor (TNF)- α in both LC and OC groups before, during and after surgery.

Methods. Sixty-one patients who underwent cholecystectomy were included in this study. This

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study was carried out at Aseer Central Hospital, Aseer region, Abha Private Hospital and the College of Medicine and Medical Sciences, King Khalid University, Abha, Kingdom of Saudi Arabia, during the time period October 1998 through to November 2000. Twenty-seven had open cholecystectomy and 34 had laparoscopic cholecystectomy. All patients were admitted to hospital one day prior to surgery where 5 ml of blood was obtained, 24 hours before surgery, on call to operation theater, and 24 hours after surgery. All patients had general anesthesia, intravenous fluids and analgesia in the first 24 hours postoperatively, and resumed oral feeding 24 hours after surgery. The data collected included the patient's age, sex, history of chronic illnesses, operative procedure undertaken and its duration and difficulties encountered, whether intraoperative cholangiogram and exploration of the common bile duct, or both were performed, and whether blood transfusion was given. The open cholecystectomy approach was through a right-sided transverse subcostal incision. The laparoscopic cholecystectomy procedure was accomplished using 2 x 10mm ports placed at the umbilicus and below the xiphisternum, and 2 x 5mm ports placed on the right midclavicular and midaxillary lines. The post operative period was observed as well as the hospital stay. Diabetic patients or those with renal failure, acute cholecystitis, or with diseases that might interfere with immune system were excluded. Five ml of peripheral blood was collected from all patients enrolled in the study before, during and 24 hours post surgery. Sera were separated, aliquoted and stored at -20° centigrade. The blood samples which were obtained from patients of both groups were used to determine serum concentrations of cytokines IL-6, IL-8, IL-1 β , and TNF- α .

Cytokines analysis. Interleukin-6. The level of IL-6 in patient's samples was measured using an enzyme-linked immunosorbent assay (ELISA) (R&D systems, Minneapolis, Minnesota (MN), United States of America (USA)). The microtiter plates (Nunc, Maxisorb, Denmark) were coated with 100 μ l of mouse anti-human IL-6 (2 μ g/ml in PBS) and incubated overnight at room temperature. The wells were washed 3 times and blocked with 300 μ l of blocking buffer (1% bovine serum albumin (BSA), 5% sucrose in phosphate buffer solution with 0.05% sodium nitrate (NaN₃). The plates were washed 3 times and 100 μ l of patients serum samples and standards were added and incubated for 2 hours at room temperature. After that the plates were washed 3 times and 100 μ l of biotinylated goat anti-human IL-6 (200 ng/ml) was added to all wells and incubated for 2 hours at room temperature. At the end of the incubation period the plates were washed and 100 μ l of streptavidin-horse radish peroxidase (HRP) (diluted 1:200) was added and incubated for 20 minutes at room temperature. After washing the

plates, 100 μ l of the substrate solution mixture (1:1 mixture of hydrogen peroxide (H₂O₂) and tetramethylbenzidine) was added to all wells for 20 minutes at room temperature. The reaction was stopped by the addition of 50 μ l of stop solution (2N sulfuric acid (H₂SO₄)) and the optical densities were read at 450 nm and corrected at 540 nm using microplate reader (ICN, titertek multiscan MCC -340 Finland). The IL-6 concentrations were calculated in pg/ml from a standard curve using computer software.

Interleukin-8. The levels of IL-8 in serum samples were determined using commercial ELISA kits (R&D systems Minneapolis, MN, USA). The same procedure used for determination of IL-6 was used but the plates were coated with 100 μ l of mouse anti-human IL-8 (4 μ g/ml) and the detection antibody was biotinylated goat anti-human IL-8 (20 ng/ml).

Interleukin 1 β . Serum levels of IL-1 β were measured using an ELISA test (Genzyme, Cambridge, Massachusetts (MA), USA). Some 100 μ l of standards and patients sera were added to microtiter plate wells coated with immobilized mouse monoclonal anti-human IL-1 β and incubated for 30 minutes at 37 $^{\circ}$ C. At the end of the incubation period the plates were washed 5 times and 100 μ l of rabbit polyclonal anti-human IL-1 β was added to all wells and incubated for 30 minutes at 37 $^{\circ}$ C. After washing the plates 5 times, 100 μ l of goat anti-rabbit immunoglobulin G (IgG) horseradish peroxidase conjugate was added to all wells and the plates were incubated for 15 minutes at 37 $^{\circ}$ C. The plates were washed 5 times and 100 μ l of hydrogen peroxide-tetramethylbenzidine mixture was added and incubated for 30 minutes at room temperature. The reaction was stopped by the addition of 50 μ l of IM H₂SO₄ and the optical densities were read at 450nm and the readings were corrected at 620nm. The concentrations of IL-1 β in pg/ml were obtained from a standard curve made by the ELISA reader. The sensitivity of the ELISA for IL-1 β is 3.0 pg/ml.

Tumor necrosis factor- α . The levels of human tumor necrosis factor alpha (hTNF- α) were measured using ELISA test (Genzyme, Cambridge, MA, USA). In brief the wells of micro titer plates (Nunc, Maxisorp, Denmark) were coated with 100 μ l of mouse anti-human TNF- α (2.0 mg/ml in 0.1M carbonate, pH 9.6) and incubated overnight at 2-8 $^{\circ}$ C. The plates were washed 5 times and the wells were blocked by the addition of 250 μ l blocking buffer (1% BSA in 0.01M PBS pH 7.3). The blocking buffer was aspirated and 100 μ l of serum samples and standards were added to the wells and incubated for one hour at 37 $^{\circ}$ C. The plates were washed 5 times and 100 μ l of rabbit anti-human TNF- α biotinylated antibody (0.5 μ g/ml in PBS with 0.5% between 20 and 1% BSA) was added to each well and incubated for one hour at

37°C. The plates were then washed 5 times and 100 ul of streptavidin horseradish peroxidase was added to all wells and incubated for 15 minutes at 37°C. Finally, the wells were washed 5 times and 100ul of hydrogen peroxide tetramethylbenzidine mixture was added to all wells and incubated for 10 minutes at room temperature. After that the reaction was stopped by adding 100 ul of 2NH₂SO₄, the absorbency was read at 450nm and corrected at 620nm. The concentrations of hTNF-α in pg/ml in patient's sera were calculated from a standard curve made by the ELISA reader. The sensitivity of the ELISA test for hTNF-α is 0.4 pg/ml.

Statistical analysis. The data were entered and analyzed by statistical package for social sciences package version 7.5. Values of IL-6, IL-8, IL-1β and TNF-α were tested for normality. The values were not normally distributed and were transformed into their log values. The significance of the levels of each cytokine and the percentage of changes following surgery were analyzed using paired t-test. Comparison between different time periods was carried out for each group separately using the paired t-test. Comparison between both patient groups was achieved by using student's independent (unpaired) t-test. The percent change was calculated to express the change of different periods as a percentage in relation to its baseline. The equation used was as follows: % Change = [(A-B)/B] x 100. Where A = log value at the following period, B = log value at the preceding period. This was calculated 3 times: baseline in relation to during and 24 hours postoperatively as well as at surgery versus 24 hours postoperatively. Independent t-test was used for comparison between the 2 studied procedures regarding the % change of log values.

Results. Sixty-one patients were entered in the study, 27 of them had OC and 34 had LC. Blood samples collected before, at and 24 hours post surgeries were analyzed for IL-6, IL-1β, TNF-α and IL-8 cytokine levels. There was significant increase in the level of IL-6 at 24 hours post surgery in the OC group compared with the LC group (p<0.04) **Table 1.** No significant changes were seen in the levels of the other cytokines (IL-1β, TNF-α and IL-8) following surgical intervention in both groups. The IL-6 levels of the 2 groups of patients before, at or 24 hours post surgery are shown in **Figure 1.** There was a significant increase in the IL-6 level within each group following surgery (P < 0.001). However, these changes were only significant between the 2 groups at 24 hours post surgery, as the mean log of IL-6 of the LC group (0.81 ± 0.70) was significantly lower than the OC group (1.12 ± 0.46) (P < 0.04) as shown in **Table 1.** A significant number of patients in the OC group responded with high levels (>5 pg/ml) of IL-6 following surgery

Table 1 - Comparison between both surgical techniques according to Interleukin-6 before, during and 24 hours post-operative.

Interleukin 6	OC (N=27) X ± S	LC (N=34) X ± S	t-value
IL-6-1 before (absolute)	24.55 ± 104.59	0.37 ± 0.59	1.201 NS
IL-6-1 log before (log)	0.16 ± 0.81	-0.09 ± 0.27	1.553 NS
IL-6-2 during (absolute)	6.76 ± 19.56	0.73 ± 1.15	1.599 NS
IL-6-2 log during (log)	0.14 ± 0.68	-0.04 ± 0.35	1.292 NS
Paired t before and during	0.129 NS	0.748 NS	0.765 NS
IL-6-3 (24 hours post) absolute	22.99 ± 27.68	32.80 ± 95.02	0.518 NS
IL-6-3 (24 hours post) (log)	1.12 ± 0.46	0.81 ± 0.70	2.016 P = 0.039
Paired t before 24 hours	5.847 P<0.001	70988 P<0.001	2.276 NS
Paired t during and 24 hours post	-6.837 P<0.001	6.420 P<0.001	0.055 NS

IL - Interleukin
 LC - laparoscopic cholecystectomy, OC - open cholecystectomy
 N - number
 NS - not significant, SD - standard deviation
 t - value calculated for % change of the log values at the corresponding time interval

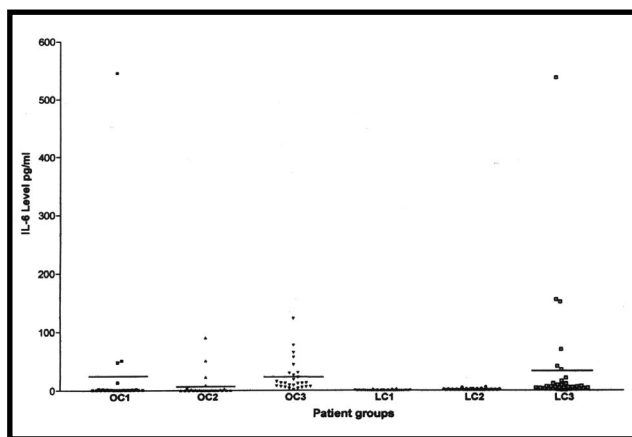


Figure 1 - Interleukin -6 level of open cholecystectomy and laparoscopic cholecystectomy patients before, at and 24 hours post surgery. IL - interleukin, OC1 - open cholecystectomy patients 24 hours before surgery, OC2 - open cholecystectomy patients at surgery, OC3 - open cholecystectomy patients 24 hours post surgery, LC1 - laparoscopic cholecystectomy patients 24 hours before surgery, LC2 - laparoscopic cholecystectomy patients at surgery, LC3 - laparoscopic cholecystectomy patients 24 hours post surgery.

Table 2 - Percentage of patients positive (>5pg/ml) and negative (<5pg/ml) for Interleukin -6 levels.

Parameter	N (%) of positive and negative patients	
	Positive	Negative
Open cholecystectomy (OC) (N=27)		
Before surgery	4 (14.8%)	23 (85.2%)
At surgery	4 (14.8%)	23 (85.2%)
After surgery	23 (85.2%)	4 (14.8%)
Laparoscopic cholecystectomy (LC) (N=34)		
Before surgery	-	34 (100%)
At surgery	1 (2.9%)	33 (97.1%)
After surgery	16 (47.05%)	18 (53%)
N - number		

compared to the LC group **Table 2**. Twenty-three out of 27 (85.2%) of the OC patients became positive for IL-6 level (> 5 pg/ml) following surgery while 16 out of 34 (47%) of the LC group became positive for IL-6 post surgery **Table 2**.

Discussion. Laparoscopic cholecystectomy, a minimal invasive surgery is fast replacing OC due to reduced surgical trauma, stress and shorter period of hospitalization.⁵ The degree of stress and trauma associated with surgical intervention are related to neurological, metabolic, and inflammatory processes following surgery. The early stress and metabolic changes are the result of the early immune mechanisms related to tissue damage and repair.⁶ In particular, surgical trauma is associated with inflammatory cytokines induced or secreted following surgical intervention. Among these inflammatory cytokines, IL-1 β , TNF- α , IL-6 and IL-8 could play an important role in the early phase of surgical trauma. We recently introduced LC for uncomplicated gall stones in replacement of the conventional OC and we assessed the level of inflammation induced by each surgical procedure by measuring the blood levels of inflammatory cytokines in LC versus OC patients before, during and after surgical intervention.

Our study, like a number of recent studies, was limited to measuring the cytokine blood levels before, during and 24 hours post surgery, mainly due to the short hospitalization period of the patients in the LC group and the lack of compliance to follow

up. Most of the studies found that major metabolic and immunological changes occur in the first 48 hours post surgery.⁷ The early changes are probably related to the induction of these cytokines, which in turn determine and control the level of stress and associated inflammation following interventional surgery.^{7,8} We found that LC surgical intervention compared to conventional OC was associated with significantly less IL-6 level following surgery (p< 0.04). Many other investigators observed the same.^{9,10} We did not find any changes in the levels of IL-1 β , TNF- α , or IL-8 in both of our study groups before, during or 24 hours post surgery. In our study, irrespective of the increase in the IL-6 levels in the OC group, we did not find any significant production or increase in the IL-1 β or TNF- α levels. This may indicate that at early hours post surgical intervention the greater induced trauma in the OC group is associated with an initial increase in the IL-6 which, at a later period could lead to the induction of TNF- α or other cytokines. Our study was limited in the duration of follow-up being only 24 hours post surgery, and may be changes in these other cytokines take a longer period to occur. The high levels of IL-6 we observed in the OC group is related to the degree of trauma following the surgery. It has been shown that the production of inflammatory cytokines is a direct affect of surgery and not the anesthesia¹¹ and that minimal invasive surgery for cholelithiasis induces an attenuated acute phase response.¹² Trauma induced inflammatory response was found to be significantly less following LC compared to conventional cholecystectomy and the inflammatory response was related to the degree of trauma.¹³

In conclusion, minimal invasive surgery is a preferable choice for the treatment of patients with uncomplicated cholelithiasis and indeed it was associated with lower levels of the proinflammatory cytokine (IL-6) compared to OC.

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