Interleukin 6 (Il_6) In Patients With Compensated Cirrhosis And Symptomatic Gall Stones After Laparoscopic And Open Cholecystectomy

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ABSTRACT: The advantages of laparoscopic cholecystcytomy (LC) for most patients have been extensively published. However its benefits and successful use in patients with cirrhosis are less documented. The study compromised fifty patients with symptomatic gallstone cholecystectomy disease and undergone either open cholecytectomy (OC) or laparoscopic. These patients were randomized into two groups: Group I included 24 patients who underwent OC, and group II included 26 patients who underwent LC. Patient age, sex, clinical presentation and child-Turcotte-Pugh (CTP) class were documented. No patients in this study had CTP class cirrhosis. IL-6 measurement by ELISA post operative, operative time, postoperative pain, hospital stay, blood loss, morbidity, recovery time, and liver function test abnormalities. There was no operative mortality. Conversion to OC was necessary in 3 patients. Mean surgical time was significantly longer in OC group (group I) than LC group (group II), (mean $\pm$ SD, 96.6 $\pm$ 32, minutes Vs 58.7 $\pm$ 23.8 minutes, P=0.037). No patients in group II required any blood replacement in contrast to 9 patients (37.5%) in group I. Intraoperative bleeding remained significantly higher in group I (P=0.043). No patients in group II had wound complications compared with 5 patients (29.14%) in group I. The group I had significantly longer hospital stay than group II, mean 9.0 $\pm$ 1.3 days (median 7) Vs 2.3 days $\pm$1.9 (median 2.5); P=0.001, lowered level of IL-6 at 6^{th} hour and 12^{th} hour post operative. Our results demonstrate that laparoscopic cholecystectomy can be performed safely in patients with CTP class A and B cirrhosis. It offers several advantages over open cholecystectomy, including lower morbidity, shorter operative time, and reduced hospital stay with less need for blood transfusions. IL-6 more significantly, increase post operatively more in open cholecystectomy than laparoscopic one as it cooperates with intensity of operative trauma.

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INTRODUCTION

Hepatitis C virus (HCV) infection affects an estimated 170 million individuals worldwide, and 5 million in the United States, where it is currently recognized as the most prevalent blood-borne infection and the leading indication for a liver transplant[(1,2)]. Treatment of HCV with pegylated interferon-α-2a is successful in iradicating virus from only 30%-80% of those treated, with individuals infected with the more resilient genotype-1 virus having markedly lower response rates than those with non-genotype-1 infections. Additionally, differences in outcome have been described by race, with African Americans having significantly lower response rates than Caucasian Americans[(3,4,5)]. Expressed in a number of different cell types, including, hepatocytes, macrophages, B-cells and T-cells, Interleukin-6 (IL-6) is a pleiotropic cytokine important in the immunologic response to infections. IL-6 plays an important role in HCV infection as well as the response to IFN therapy. In addition to interacting with crucial components of the interferon response pathways, IL-6 is an activator of acute phase proteins in hepatocyes. A recent study has suggested the potential importance of IL-6 in the treatment response of HCV patients to interferon-based therapy[(6)]. It has been reported that the degree of post operative inflammation is reduced after laparoscopic surgery. Other groups also observed significantly better preservation of lymphocytes subpopulations, neutrophil function, and cell-mediated immunity after laparoscopic vs open colorectal surgery. Further more, it has been observed that cell-mediated immunity, as assessed by delayed-type hypersensitivity testing in humans, is better preserved after laparoscopic vs open colorectal resection. This lesser degree of operative stress was also confirmed by experimental animal studies by *Matthias et al*[(7)]. Infection with hepatitis C virus (HCV) has become the most important public health problem in Egypt. There is a high incidence of anti-HCV seropositivity among the Egyptian population, with an overall age-adjusted prevalence of HCV antibodies of 21.9%[(8)]. Schistosoma mansoni (SM) is endemic in Egypt. The prevalence of Schistosoma mansoni in the Nile River Delta extends

to 45% of the population[9]. Chronic viral hepatitis C and infection with SM are the two major causes of chronic liver disease in Egypt[10]. Gallstone are twice as prevalent in patients with cirrhosis than in the general population[11]. Factors implicated in the higher incidence of gall stone formation include hypersplenism, increased levels of estrogen, and increased intravascular haemolysis with reduction in gall bladder emptying and motility[12,13]. The technical challenge and risk of performing open cholecystectomy (OC) in patients with compensated cirrhosis and symptomatic gall stone disease have been well documented[14,15]. The severity of cirrhosis assessed with child-Turcotte-Pugh (CTP)classification, is a major determinant in deciding which treatment approach is optimal. Since the introduction of laparoscopic (LC), a controversy regarding which procedure is preferable for patients with cirrhosis has arisen[16,17]. Several studies reported good results and suggested liberal use of LC in patients with early-stage cirrhosis and symptomatic gall stone disease [18,19]. No definite data have been offered to prove LC should be the first surgical line in patients with cirrhosis[20]. The present study is a prospective analysis comparing the results of OC and LC in patients with compensated cirrhosis and symptomatic gall stone disease. The purpose of this study was to compare the risks and benefits of performing open cholecystectomy (OC) and laparoscopic cholecystectomy (LC) in patients with compensated cirrhosis, and role of IL-6 post operative.

PATIENTS and METHODS

This study included 50 patients with hepatic cirrhosis and symptomatic gall stone disease underwent cholecystectomy at Mansoura University Hospital, Mansoura Egypt. These patients were subjected to a thorough history and clinical examination focused on manifestation of gall stone disease and chronic liver disease. The following laboratory investigation were performed: Urine and stool analysis; Liver function tests (serum bilirubin, ALT, AST, prothrombin time, INR and serum albumin); Whole blood picture; Kidney function tests; HCV and HBV markers. The diagnosis of schistomiasis was based upon positive stool analysis or positive rectal snip for schistosomal egg. A history of repeated antischistosomal treatment and/or positive indirect hemagglutination assay (IHA) for schistomiasis indicated previous exposure[21]. Patients positive for schistosoma mansoni (SM) with negative viral markers were considered pure SM. Patients with positive anti HCV antibodies, high liver enzymes, positive HCV by RT-PCR, and negative for schistomiasis were assigned to the pure HCV group. Patients positive for both HCV and schistomiasis made up the mixed (HCV+S) group. Patients positive for HBV and negative for HCV are considered pure HBV group. Patients positive for both HCV and HBV made up the mixed (HCV + HBV) group. HCV-RNA analysis were done on serum samples that had been stored at -70^{O}C immediately after collection. In brief, extracted RNA was converted into cDNA that was used as a template for PCR amplification using primer sequences derived from highly conserved 5\- non coding region of the genome (outer primers: sense 5\-CCA TGG CGT TAG TAT GAG TG-3, anti-sense 5\ TGC TCA TGG TGC ACG GTC TA-3 and inner primers: sense 5\- AGA GCC ATA GTG TGC GG-3\, anti-sense 5\-CTT TCG CGA CCC AAC ACT AC-3\.) HCV genotypes for all patients were performed by reverse transcription polymerase chain reaction (RT-PCR) using specific primers[22]. The typing assay is based on a first outer PCR utilizing consensus primers to amplify a 284 bp segment of the HCV core gene. Thereafter 1.0ml of the first PCR product is used as a target in a second PCR with one consensus upstream primer and four type specific downstream primers, each giving a product of a type specific size[23]. Genotypes of HCV were distinguished from each other by size of product: 52 bp for type I, 141 bp for type II, 173 bp for type III, and 126 bp for type IV. The diagnosis of cirrhosis had been proven on clinical basis, laboratory results, macroscopic intra-operative appearance and liver biopsy (15 patients preoperative and 13 patients intra-operative). The prepared slides were examined by a pathologist to detect and stage fibrosis as *Knodel et al*[24], and the histopathologic activity index based on the assessment of portal inflammatory infiltrate, interface hepatitis and parenchymal necrosis (Fig 1,2,3 & 4). The diagnosis of gall bladder lithiasis had been determined in all patients by clinical history and abdominal ultrasonography. Pathological processing for liver biopsy specimen was as follows: 4mm sections were prepared from paraffin blocks and stained for hematoxyline and eosin stain, masson trichrom. Reticulin, PAS and PAS-diastase stains. HBsAg and HBcAg immunochemistry were applied routinely to all liver biopsy specimens. Monoclonal antibody to HBsAg and polyclonal antibody for HBcAg were applied (Zymed, South San Francisco, CA). Any positive cases for HBV were excluded. Prepared slides from liver biopsy specimen were examined blindly by the pathologist. Degree of necroinflammatory injury and stage of fibrosis were assessed according to *Knodell et al*[24], however, with modification. Histologic activity index (HAI) is based upon assessment of portal inflammatory infiltrate, interface hepatitis and parenchymal necrosis, with score range from 0-18. it was considered as minimal (1-3), mild (4-8), moderate (9-12) and severe (13-18). Fibrosis is staged separately on a scale of 0-4, corresponding to absent fibrosis up to cirrhosis. Randomization was carried out using presealed

envelops immediately before the surgery. The child-Turcotte-Pugh classification system was used to assess the severity of cirrhosis. No patients in this study had a CTP class C cirrhosis. All operations were performed under general anesthesia; hepatotoxic drugs were avoided. Estimated intra-operative bleeding was recorded from surgical reports, and blood loss was classified as less than 200mL, 200 to 500mL, and more than 500mL. Blood replacement was also documented. Surgical and anesthetic times were documented as were intraoperative findings, perioperative complications, and length of hospital stay. A standard laparoscopic procedure was used for all patients, as described by *Doubois et al*[25]. Conventional cholecystectomy employed a 13cm subcostal incision (10 patients) or upper right paramedian incision (14 patients). Post operative subjective pain score on mobilization using the VAS (Visual analogue scale) was recorded on the 1st, 3rd, 7th post operative days[26]. Also the serum interleukin-6 was measured at the 6 hour, and the 12 hour, post operative using immunoassay. IL-6 assay by ELISA (Titrezyme perspective biosystem) 2ml of blood sample was withdrawn from all subjects under sterile condition, centrifuged at 3000rpm for 15 minutes and the non hemolyzed serum was separated and preserved at -70°C till cytokine assay. Circulating serum IL-6 level was determined using ELISA Assays as described by the manufacturer. The patients are followed up weekly for the 1st month, monthly for the first three months and every three months for a range of 12 months to 36 months, clinically, laboratory (liver function test) and radiological (abdominal ultrasound). Statistical comparison between the OC and LC groups were made with Fisher's exact test for variables with continuous or ordinal distribution.

RESULTS

The patient demographic data, and clinical presentation of gall bladder disease, etiology of liver disease, and its severity are shown sequential (tables 1, 2 & 3). Three cases were converted from laparoscopic to open cholecystectomy (one patient due to dense vascular adhesions, patients due to difficult dissection of Calôts triangle and another patient due to uncontrollable liver bed bleeding). (Table 4): showed shortened surgical time and hospital stay for LC group in comparison to OC group (P<0.05). There was a significant intraoperative blood loss in OC group (P<0.05). Ten patients necessitating blood transfusion ranging from 1-2 units, but the estimated post operative blood loss was statistically insignificant between both groups (Table 5). Table (6) showed a significant reduced pain (subjective) score in LC group and similarly less burden on the patients metabolic response as reflected by lowered IL-6 level at the 6th hour and the 12th hour post operative (P<0.05). No operative mortality in both groups. Lastly table (7) showed patients morbidity which is more frequent in OC group than LC group (P<0.05), with statistically significant difference as regard readmission in OC group (three patients with encephalopathy, one patient with ascetic fluid leak, one patient with wound hemorrhage, and one patient with wound infection necessitating debridment) and deterioration of liver function in OC group. Although bile leakage was more common in LC group (11.5%) but it showed no significant value.

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Table (1): *patient's demographics data*

Patients	OC group		LC group		P value
	n (24)	%	n (26)	%	
Age					
Mean ± SD	42.3 ± 15.3		40.6 ± 14.1		NS*
Sex					
Male	10	41.7	10	38.5	NS
Female	14	58.3	16	61.5	
Clinical presentation					
Acute cholecystitis	2.0	8.3	1	3.8	NS
Biliary colic	22.0	91.7	25	96.2	

*Non-significant

Table (2): *Etiology of liver disease*

Etiology	OC group		LC group	
	n (24)	%	n (26)	%
Mixed HBV & HCV	3	12.5	3	11.54
Pure HBV	1	4.17	2	7.69
Pure schistosomal	3	12.5	4	15.38
Pure HCV	9	37.5	11	42.31
Mixed schistosomal & HCV	7	29.17	7	26.92

Table (3): *Severity of hepatic cirrhosis*

CTP	OC group		LC group	
	n (24)	%	n (26)	%
A	17	71	22	85
B	7	29	4	15
C	0	0	0	0

Table (4): *Surgical time and hospital stay*

	OC group n(24)	LC group n(26)	P value
Surgical time (min)			
Mean ± SD	96.6 ± 32	58.7 ± 23.8	<0.05
Range	50.0 – 137	47 – 116	
Hospital stay (day)			
Mean ± SD	9.0 ± 1.3	2.3 ± 1.9	<0.05
Range	3 – 7	1 – 8	

Table (5): *Intra & post operative blood loss (mL)*

	OC group		LC group		P value
	n (24)	%	n (26)	%	
Intra operative loss					
< 200	9	37.5	20	76.9	<0.05
200 – 500	6	25	4	15.4	
>500	9	37.5	2	7.7	
Blood transfusion					
Yes	10	41.7	0	0.0%	<0.05
No	14	58.3	26	100	
Post operative loss					
< 200	20	83.33	25	96.15	NS*
200 – 500	2	8.3	1	3.84	
> 500	2	8.3	0	0	

*non significant

Table (6): *Post operative pain and interleukin-6 (U/mL)*

	OC group		LC group		P value
	Mean	±SD	Mean	±SD	
Post operative pain					
1st day	8.7	0.77	3.9	0.41	<0.05
3rd day	5.46	0.47	2.1	0.35	<0.05
7th day	4.41	0.73	1.3	0.48	<0.05
Post operative IL-6	Mean	Range	Mean	Range	
6th hour	1150	1250 - 750	450	550 – 450	<0.05
12th hour	900	1100 - 700	400	500 – 320	<0.05

Table (7): *Patients morbidity*

	OC group		LC group		P value
	n (24)	%	n (26)	%	
Morbidity	16	60	8	30.7	< 0.05
Pulmonary Infection	3	12.5	1	3.8	NS*
Urine retention	2	8.3	0	0	NS
Deterioration of liver	8	40	4	15.4	< 0.05
function	4	16.7	1	3.8	NS
Ascitic fluid leak	1	4.2	4	11.5	NS
Bile leakage	1	4.2	0	0	NS
Encephalopathy					
Wound complications					
Hemorrhage	2	8.3	1	3.8	NS
Infection	5	20.8	0	0	
Readmission	6	25	0	0	< 0.5
Incisional hernia	4	16.7	0	0	< 0.5

Figure (1) A case of chronic hepatitis with marked activity and cirrhosis showing interface hepatitis with marked lymphocytic infiltration (H & E x40)

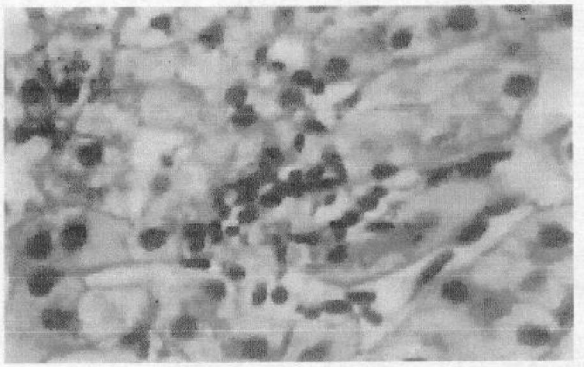

Figure (2) A case of hepatitis C showing focal liver cell necrosis replaced by inflammatory cells (H & E x100)

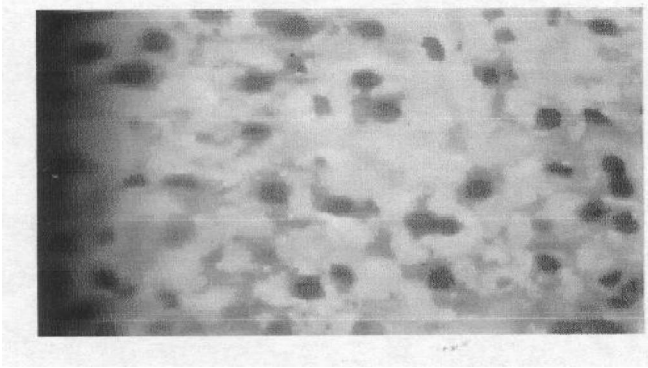

Figure (3) A case of hepatitis B showing ground glass hepatocytes (H & E x 100)

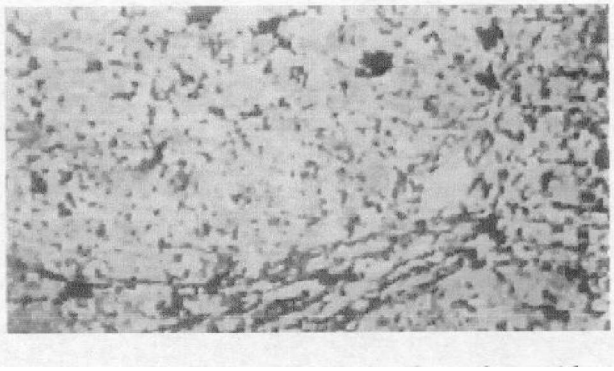

Figure (4) Liver biopsy with Schistosomal granuloma with central bilharzial ovum in the portal area (H & E x 100).

DISCUSSION

Chronic liver disease represents a major cause of morbidity and mortality worldwide. The major etiologies are chronic infection with hepatitis B (HBV) and C (HCV) viruses, and alcoholic and non-alcoholic fatty liver disease. Chronic hepatitis B and C are the leading causes of cirrhosis and of hepatocellular carcinoma worldwide. Approximately 400 million people are chronically infected with HBV and 25%-40% of them die of cirrhosis and of its end-stage complications[27]. HBV is the most important carcinogen after tobacco and the incidence of hepatocellular carcinoma is 300,000 cases per year[28]. Chronic hepatitis C is a major health concern with around 200 million individuals affected worldwide, with a greater prevalence in Western countries. Natural history studies indicates that advanced fibrosis and cirrhosis develop in about 20%-40% of patients with chronic viral hepatitis[13]. Since the first laparoscopic hepatic lobectomy performed by Reich in 1991, the laparoscopic operation procedure has been practiced in the hepatobilliary surgery[17,29]. Because of the abundant blood supply for the liver and pneumoperitoneum, hemorrhage and gas embolism often occur during operation[30]. Laparoscopic hepatic lobectomy is an operation procedure with high difficulties and risks[31]. However, in recent years, with the development of laparoscopic instrument and operative skills, laparoscopic hepatic lobectomy has gradually become a common operation procedure in clinical practice[29,32]. It has many advantages over the traditional open operation. In traditional open operation, T-pieces should be placed in the common bile duct to drain the bile after exploration of the common bile duct, which provides channels for surgeon to take out the possible remnant gallstones. However, it also bring about heavy burdens on the patients, such as prolonged hospitalization time, unbalance between electrolytes and digestive dysfunction caused by the T-piece, complicated care for the T-piece. Research have tried to solve these problem for a long time. In the early 1980s, open cholecystectomy in patients with cirrhosis was associated with a post operative mortality ranging from 7% to 26%[33]. Excessive blood loss, post operative liver failure, and sepsis were responsible for most of these deaths[34]. The importance of stratifying patients according to CTP criteria as a method to predict perioperative complications was clearly demonstrated. By the late 1980s better surgical results had been published for cirrhotic patients with normal hepatic synthetic function who underwent elective cholecystectomy[11,14]. Open cholecystectomy was subsequently considered an acceptable therapeutic option in patients with CTP class A and B cirrhosis. If the patient had class C cirrhosis and symptomatic gallstone disease, attempts were made to improve the patient's hepatic function and control of ascitis to allow for a safer elective operation[35,36]. Since the introduction of LC, question of whether cirrhotic patients might benefit from this less invasive approach has been arisen, but not fully answered. Several recent studies[15,36] have demonstrated that LC in child A and B cirrhosis was safer and better tolerable than OC. There were less incision-related complications, no mortality, low morbidity, shorter operative time and hospital stay and fewer transfusion requirements[19,20]. In this study the absence of mortality and low morbidity in the laparoscopic group confirm the privileged indication of laparoscopic cholecystectomy in cirrhotic patients, also the low conversion rate similar non cirrhotic patients narrow the spectrum of conversion and enhance laparoscopist to maintain that low conversion threshold. These data also confirmed by *Marino et al*[36]. Also the shortened surgical time of laparoscopic group with meticulous surgical procedure and the shortened hospital stay is related to the less morbidity and better local and systemic responses (physiologic, immunologic and metabolic) to this minimal invasive surgery[19]. In this study the reduced blood loss in the laparoscopic group, whether operative or postoperative, is related to the meticulous dissection (magnified surgical field) and the pneumoperitoneum barohaemostatic effect. These data correlate with the finding of *Yardel et al*[16], *Zhang et al*[20] *and Mamada et al*[37]. The serum Il-6 level postoperatively was significantly increase, more in open cholecystectomy than laparoscopic one. These data proved that the serum IL-6 correlate well with the intensity of operative trauma[38]. The low subjective pain threshold and low objective interleukin 6 levels in LC group signify less body physiologic response to this minimal invasive procedure and support its liberal use in these fragile patients as reported by *Schafer et al*[39] *and Matthias et al*[7]. Similarly the absence of intraoperative intestinal retraction and less pain in the laparoscopic group explain the low occurrence of postoperative ileus and urine retention[40]. Moreover the absence of parietal incision in the laparoscopic group declines the incidence of pulmonary complications, incisional hernia, and readmission. These findings also reported by *Poggio et al*[41]. Also the reduced bleeding, intraoperative and postoperative, minimal dissection and shorter operative time all explain the lack of deterioration in liver function[15,42]. Nevertheless the shortened hospital stay, less morbidity and absent readmission decrease the total patient costs similar to that reported by *Sleeman et al*[43]. Lastly laparoscopy as an alternative to open cholecystectomy is both beneficial to the patient and the surgeon. Laparoscopic cholecystectomy in cirrhotic patients prevents the hypervascular adhesions that contraindicate orthotopic

liver transplant[44] and lessen the contamination risk to the medical and paramedical personnel[45]. It can be concluded that, an elective LC should be considered for every patient with CTP class A or B cirrhosis and symptomatic gallstone to prevent minimized biliary tract complications and the procedure had a better outcome, shortened operative time and less hospital stay, tolerable loco-systemic body response, wide range of safety, less economic, and preserve surgeon's safety.

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