Original Article

Comparative assessment of TNF-α and C-reactive protein in patients subjected to open instead of laparoscopic cholecystectomy

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ABSTRACT

Background: C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) are specific proteins modulated by the acute stress of surgery. Various retrospective and prospective studies have demonstrated subdued stress responses in laparoscopically operated patients vis-à-vis the conventional (open) method. **Aim:** To assess the roles of TNF-α and C-reactive protein levels as tentative stress markers in correlating the extent of perioperative immune modulation during open and laparoscopic cholecystectomy in a subset of an Indian population. **Setting and Design:** This study is a prospective study. **Materials and Methods**: Forty patients admitted and operated over a period of two years, were assigned to two groups: laparascopic cholecystectomy (LC) (Group-I) or open cholecystectomy (OC) (Group-II). Selection was based on: patients' preference, patients' insistence, patients' inability to afford the laparoscopic procedure, lack of expertise or lack of facility in the hospital for the laparoscopic procedure. The baseline characteristics in terms of anesthetic protocol, fluid management, postoperative orders and perioperative care were kept similar for both groups. **Statistical Analysis:** SPSS v.11 was used for statistical computations. **Results:** Baseline values of CRP in the OC and LC Groups were 7.9959 ± 5.57837 µg/ml and 11.3948 ± 11.61055 µg/ml respectively. CRP values rose markedly during the intra- as well as the postoperative periods till day 4 ($P < 0.001$) in both the groups. Intragroup variations were not significantly different from each other ($P > 0.05$). No such trend was noticed for TNF-α levels under identical conditions. Indeed, the TNF-α levels were 2.0304 ± 5.67384 pg/ml and 11.3408 ± 25.09385 pg/ml in the OC and LC groups respectively. Intragroup changes were not significantly different from each other ($P > 0.05$). Percentage changes in CRP and TNF-α were higher after OC in comparison to LC. **Conclusions:** The present study did not record statistical differences in the levels of the two markers (CRP, TNF-α) between the two groups. This reflects the limited tissue trauma due to easy and simple visceral tissue dissection in the two types of nondifficult surgery intraoperatively. However, greater change ($P = 0.09$) of TNF levels (30% *vs.* 1.9%) in the OC group on the 4th postoperative day represents the possibility of greater immune modulation in the OC group postoperatively.

Key words: C-reactive protein, laparoscopic cholecystectomy, open cholecystectomy, stress response, TNF-α

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Surgery, the "controlled deliberate injury" for the purpose of therapy, induces a series of biochemical and hormonal responses in the body that include alterations in the expression of acute phase reactants-CRP and TNF-α. In general, the magnitude of changes in these proteins are apparently taken as proportional to the extent of overall surgical insult. The amount of visceral tissue manipulation and dissection is often not taken into account during

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different surgical techniques and emphasis is put mainly on the parietal tissue trauma although it may significantly influence the surgical stress. Laparoscopic cholecystectomy (LC) is usually considered to be a safer procedure than conventional cholecystectomy in terms of metabolic, hormonal and immunological changes.[1-3] Although factors like visceral tissue dissection, surgeon's skills, selection of patients and standardization of the surgical procedure significantly influence the stress response, they have hitherto not been largely considered. We postulated that LC induced lower stress responses than the conventional open cholecystectomy and that comparison of TNF-α and C-reactive protein (CRP) levels would aid in monitoring these stress responses. Therefore, a pilot study was conducted to test the proposed hypothesis in patients of Indian origin with uncomplicated cholelithiasis.

MATERIALS AND METHODS

Subjects

The study was approved by the Board of Studies (BOS) and Committee for Advanced Scientific Research (CASR) of our University. This study was conducted over a period of two years from October 2004 to December 2006. After detailed informed consent, elective cases (American Society of Anesthesiologists (ASA) grades I and II) with symptomatic, uncomplicated cholelithiasis (fit for laparoscopic procedure) were considered for the present study. Forty cases included in the study were divided into two groups-20 patients in each group depending upon the selection criteria. Patients with acute cholecystitis, pancreatitis, choledocholithiasis, malignancy, jaundice, history of allergy, steroid intake, cytotoxic chemotherapy, pregnancy or hypoproteinaemia and patients requiring perioperative blood transfusion were excluded from the study.

Sample size

In consultation with our statistician, the sample size was calculated taking into account the reported prevalence of stress during anesthesia and surgery. Considering the prevalence of stress in surgical patients to be 100% (from previous studies and past records), we took it as 95% and allowed an error of 5% of prevalence of stress on either side so as to correct our estimate in 95 out of 100 patients. Hence, if "p" is the prevalence of stress rate in percentage and "q" is the nonstress rate in percentage and p + q = 100, then the standard error is $\sqrt{(pq/n)}$ or $2\sqrt{(pq/n)} = 10\%$ of p where n is the sample size. Putting the value of p = 95% and q = 5% in the equation:

$(4 \times 95 \times 5)/n = (10 \times 95/100) \times (10 \times 95/100)$
The value of ***n*** would be **21.**
Following randomization according to patients' selection criteria, this study included 40 patients.

Reasons for patients undergoing open cholecystectomy (OC)

1. Financial: inability to afford laparoscopic procedure.
2. Patients' preference: for a specific general surgeon who lacked suitable expertise in the laparoscopic technique
3. Lack of facilities: *de novo* absence or malfunctioning of existing gazettes
4. Patients' insistence: patients' phobia of complications after laparoscopic cholecystectomy based on bad experience of kith and kin.
5. Lack of expertise: inavailability of laparoscopic surgeons

Sample method

Venous blood (5 ml) was withdrawn from the antecubital vein into a plain sterile glass vial. The samples were centrifuged at 3000 g for 10 min for separation of serum. The sera were transferred into plastic tubes and transported in ice boxes for storage at -60°C in the Institute of Microbiology where the biochemical analysis was done by competent laboratory personnel blinded to the study, test serum and surgical intervention given to the patients.

Sample times

Serial measurement of CRP and TNF-α was done by sampling the blood.

1. First baseline sample was collected preoperatively after overnight fasting.
2. Second sample at time of separation of gall bladder from the liver bed.
3. Third sample at the time of completion of surgery.
4. Fourth sample on 1st postoperative day.
5. Fifth sample on 2nd postoperative day.
6. Sixth sample on 4th postoperative day.

Perioperative protocol

All cases were first screened in the preanaesthetic clinic for relevant history and clinical examination. The investigational findings were recorded in the preanaesthetic cards.

All the procedures were explained to the patients who were advised to take tablet diazepam (5 mg) orally the night before the surgery.

Anesthesia

Patients received midazolam (0.04 mg/kg) intravenously in the preoperative room 30 minutes before induction of anesthesia. Subsequently, injection metoclopramide (0.2 mg/kg) and fentanyl (2 μg/kg) bolus were given as preemptive adjuvants to surgical analgesia. Inj. fentanyl was repeated 1 μg/kg/h till the surgery was completed.

All the patients were anesthetized with injection

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thiopentone sodium (6-7 mg/kg). Tracheal intubation was facilitated with the help of vecuronium bromide (0.12 mg/kg) and maintained with intermittent repeat doses of vecuronium, titrated with train-of-four response of 0-1 twitches. To maintain the systolic blood pressure within 20% of the basal level, halothane (0.5-1%) was used. The inspired oxygen concentration (FiO_2) was adjusted to 40% in nitrous oxide to maintain the saturation of peripheral oxygen (SpO_2) level above 98%. All the patients were ventilated mechanically at the rate of 12 breaths/min. Perioperative monitoring includes heart rate (HR), mean arterial blood pressure (MAP), electrocardiogram (EKG), saturation of peripheral oxygen (SpO_2) and end-tidal carbon dioxide ($ETCO_2$), which was maintained in the range of 35 to 40 mm Hg. Before reversal of anesthesia, a deep intramuscular injection of diclofenac sodium (1.5 mg/kg) was given to all patients as part of a routine anesthetic practice. The neuromuscular block was reversed with inj. neostigmine (0.05 mg/kg) and glycopyrrolate (0.01 mg/kg) at the end of the surgery.

Fluid therapy

The intravenous fluid regime consisted of 500 ml of Ringer's lactate solution and 500 ml of 5% dextrose solution during the intraoperative period. This regime was continued for 6-24 hours at the rate of 80–100 ml/hr depending upon climatic conditions. Usually oral intake was allowed in the same evening in the LC group and in the next morning in the OC group subject to patients' tolerance.

Surgery

The surgical procedures were performed by consultant surgeons. OC was performed through right subcostal incision and laparoscopic by 4-trocar technique with electrocautery dissection. Pneumoperitoneum was achieved with carbon dioxide insufflation, maintaining an intraabdominal pressure of 12-14 mm Hg. Patients who required perioperative cholangiography or conversion to laparotomy were excluded from the study.

Postoperative care

Deep intramuscular injections of diclofenac sodium 1.5 mg/kg were recommended every eight hours in the postoperative order as the analgesic protocol. On the 1st day of surgery, injection pentazocine (0.5 mg/kg) and injection phenargan (2 mg/kg) were given together intramuscularly in the night.

The intensity of pain was assessed with the help of a visual rating scale (VRS).[4] The amount of pain described by the patient as a score of 0 represented no pain, 1 = mild/slight pain, 2 = moderate pain, 3 = severe pain and 4 = represented very severe or intolerable pain. The enteral and parenteral analgesics were provided to the patient accordingly and the timing of the injections was noted in the case sheet.

Exclusion criteria

Patients with per-operative changes in their vital signs > 25% from the basal level and requiring further supplementation in the anesthetic protocol were excluded from the study.

Patients developing significant alterations in their baseline preoperative investigations (haemoglobin, total and differential leukocytes count, blood urea, serum creatinine, serum sodium and potassium, platelet count and prothrombin time) were also excluded from the study.

Any intraoperative injury to an adjacent organ / structure including bile duct injury led to exclusion of that case from the study.

Immunological assays

The quantitative determination of CRP concentration in plasma was done by a microplate immunoenzymometric assay according to the manufacturer's instructions (Product Code: BC 1119M from CALIBIOTECH INC. USA). The absorbance obtained from the microplate enzyme-linked immunosorbent assay (ELISA) reader was plotted in duplicate versus the corresponding CRP concentration in μg/ml with help of "Microbiology-Instant" software. The hs-CRP (high-sensitivity CRP) microplate ELISA procedure has a sensitivity of 0.2 μg/ml. The intraassay and interassay coefficients of variation in within-run precision for CRP were 7.5–2.3% and 4.1–2.5% respectively. The expected normal value for hs-CRP was taken as < 3 μg/ml.

Plasma concentration of human TNF-α was determined by ELISA from the kit (Diaclone Immunoassay, product no. 850 090 096). Results were read on a spectrophotometer using 450 nm as the primary wave length. Average absorbance was calculated for each set of duplicate standards, samples and controls. The linear standard curve was created by plotting the mean absorbance for each standard concentration on the Y axis against the TNF-α standard concentration on the X axis. The overall intraassay coefficient of variation was calculated to be 3.3%. The interassay coefficient of variation was 9%. Spike recovery ranged from 74-90% and the expected normal serum value was taken < 8 pg/ml.

Statistical analysis

Data was analyzed with standard computer software (SPSS v11.0). Intragroup and intergroup comparisons were done by paired sample statistics and independent sample T-tests respectively. Values were expressed as means with standard deviation (SD) when normally distributed, or medians with the 25th and 75th percentile interquartile range, when skewed. Nonparametric tests (Mann-Whitney U Test, Wilcoxon signed ranks

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test, Kruskal Wallis test and Jonckheere-Terpstra test) were used to compute the differences between the two study groups with respect to serial sampling. The changes within the groups were evaluated by nonparametric tests. Two-tailed P values were reported and a probability value of $P < 0.05$ was considered statistically significant.

RESULTS

In the study period from October 2004 to December 2006, 406 patients underwent cholecystectomy although only 40 cases could enroll in the study due to our exclusion criteria. Patients included in the study had comparable baseline characteristics (age, sex, weight, height, type and duration of surgery and duration of anaesthesia) [Table 1]. The medians of CRP and TNF-α with their 25th and 75th percentile interquartile range were found to be normally distributed and hence, have not been documented here. The mean ± SD of CRP and TNF-α for both LC and OC groups are given in Tables 2 and 3 and graphically represented in Figures 1 and 2. Serial percentage changes in CRP and TNF-α in both the groups are given in Table 4. A marked increase ($P < 0.001$) in CRP levels was observed in OC and LC groups during the intra- as well as postoperative periods till day 4. However, intergroup differences were not significantly different ($P > 0.05$) from each other [Table 2]. In both the groups, the increased serum CRP concentrations reached the maximum level 24-48 hours after surgery and started declining from the 4th day after surgery [Figure 1].

The values of TNF-α in both the groups did not differ significantly ($P > 0.05$) during the intraoperative period. Postoperatively its values rose markedly after the surgery in the OC group from the 2nd to 4th day ($P < 0.01$). However, intergroup changes in TNF-α were not significantly different ($P > 0.05$) from each other in the peri-operative period [Table 3].

Independent sample T-test for equality of means by application of Levene's test for equality of variances and the 95% confidence interval of the mean difference (CID) were comparable in both the groups. When the results were compared between the two groups using the Mann-Whitney U test and the Wilcoxon W test, the two-tailed significance values were > 0.05. The response of CRP following surgery was not correlated ($P > 0.05$) to changes in TNF-α as the ties between the two markers on application of Bivariate correlation by Pearson test and Spearman test was found to be insignificant.

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Table 1: Comparison of demographic data (mean ± SD) in both the groups

Variables (Mean ± SD)	Open cholecystectomy	Laparoscopic cholecystectomy	*P* value
Age in years	33 ± 5	31 ± 5	> 0.05
Weight in kg	46 ± 7 kg	45 ± 8 kg	> 0.05
Height (cm)	156 ± 4	155 ± 6	> 0.05
Sex (*n*, M/F)	0/21	0/21	> 0.05
Duration of surgery (min)	73 ± 9	71 ± 4	> 0.05
Duration of anaesthesia (min)	86 ± 4	83 ± 5	> 0.05

Table 2: C-reactive protein (μg/ml) mean ± SD with *P* values in patients undergoing laparascopic cholecystectomy and open cholecystectomy

Variables	LC (Mean ± SD)	*P* value	OC (Mean ± SD)	*P* value	OC *vs.* LC *P* value
Preop	11.3948 ± 11.61055		7.9959 ± 5.57837		0.862
Dissection of GB	15.8826 ± 13.39302	0.212	9.7660 ± 5.13915	> 0.001	0.698
End of surgery	17.1596 ± 11.94176	0.011	18.3807 ± 11.8174	< 0.001	0.738
1st postop day	38.4033 ± 14.82422	< 0.001	44.5418 ± 4.95632	< 0.001	0.698
2nd postop day	46.0838 ± 3.27104	< 0.001	47.2053 ± 4.7942	< 0.001	0.414
4th postop day	40.4195 ± 7.90892	< 0.001	42.9437 ± 5.70931	< 0.001	0.121

GB - Gall bladder, LC- Laparascopic cholecystectomy, OC - Open cholecystectomy

Table 3: TNF-α (pg/ml) mean ± SD with *P* values in patients undergoing laparascopic cholecystectomy and open cholecystectomy

Variables	LC (Mean ± SD)	*P* value	OC (Mean ± SD)	*P* value	OC vs. LC (*P* value)
Preop	11.3408 ± 25.09385		2.0304 ± 5.67384		0.740
Dissection of GB	10.2322 ± 28.80873	0.500	0.2795 ± 0.89687	0.063	0.301
End of surgery	11.4041 ± 25.06699	0.917	0.6510 ± 2.09292	0.237	0.301
1st postop day	4.9271 ± 14.03764	0.345	17.7762 ± 41.55977	0.069	0.381
2nd postop day	19.7009 ± 51.71799	0.594	27.2540 ± 44.09036	0.008	0.428
4th postop day	22.7927 ± 70.50013	0.779	59.9436 ± 174.29275	0.008	0.094

GB - Gall bladder, LC- Laparascopic cholecystectomy, OC - Open cholecystectomy

DISCUSSION

Surgical intervention, a form of deliberate trauma, is followed by well-documented inflammatory, hormonal and immunological responses that may be the deciding factors for the final outcome of the patient.[3,5] Laparoscopic surgery is often perceived as minimally invasive surgery with attenuated responses and this perception has been proved in many studies.[6]

However, the majority of these studies fail to specifically address the impact of visceral tissue dissection that may also noticeably affect the neuroendocrine metabolic responses, depending upon whether the cholecystectomy is straightforward easy or difficult.

Our observations on the comparative assessment of TNF-α and CRPs in patients subjected to open instead of LC, ($P > 0.05$) were consistent with those of McMahon and associates.[5] McMahon *et al*. evaluated several markers including CRP following LC and minilaparotomy cholecystectomy. Redmond and colleagues[7] were also unable to detect any significant difference in CRP levels between open and LC. However, they did observe enhanced TNF expression after open cholecystectomy.

These results may imply that the amount of visceral tissue dissection and tissue injury may be an important contributory factor towards systemic immune responses. This aspect may be the true reflection of LC where the patient is invariably selected on the technical feasibility based on preoperative imaging studies. This means that the similar trauma response in both LC and OC groups may be the effect of patient selection criteria. This is because added tissue dissection in complicated cases may enhance the degree of immunological modulation many fold, an observation that was also confirmed by others.[8,9]

Skin incision is frequently the site of maximum tissue trauma and is accordingly assumed to evoke greater acute phase responses. However, in a prospective randomized study (n = 34), Hill and associates[10] did not find any significant difference in CRP and Interleukin-6 (IL-6) expression following open and laparoscopic inguinal hernia repair that involves only parietal tissue trauma in either approach.

In an animal model study, Douglas *et al*.[8] and later on, Bessler and associates[9] have observed greater elevations in IL-6 after laparoscopic colectomy in comparison to open colectomy. However, higher percentage increases in TNF levels in the OC group on the 4th postoperative day suggest that LC and OC are equally stressful intraoperatively but subsist less in the

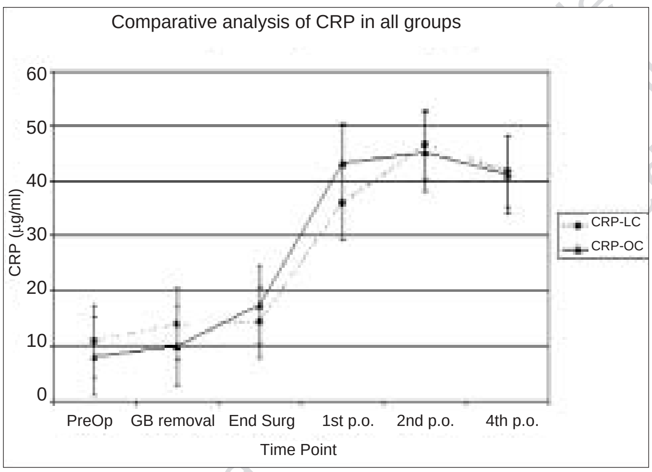

Figure 1: Perioperative changes in plasma CRP concentrations (Y axis) in the LC group (n = 20) and OC group (n = 20). X axis denotes measurements at various sampling times.

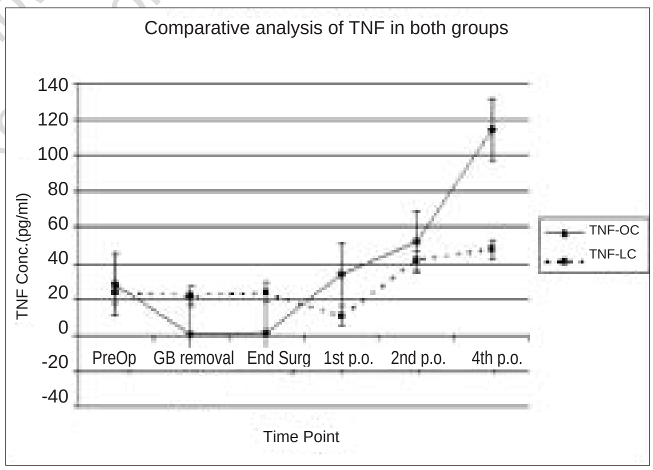

Figure 2: Perioperative changes in plasma TNF-α concentrations (Y axis) in the LC group (n = 20) and OC group (n = 20). X axis denotes measurements at various sampling times.

Table 4: Percentage changes in C-reactive protein and TNF-α levels in laparoscopic cholecystectomy and open cholecystectomy groups at various sampling times

% change	At GB dissection		End of surgery		1st postop day		2nd postop day		4th postop day	
operation	LC	OC	LC	OC	LC	OC	LC	OC	LC	OC
C-reactive protein	1.3	1.1	1.5	2.3	3.5	5.9	4	6	3.6	5.3
TNF-α	0.9	0.1	1	0.3	0.4	9	1.6	14	1.9	30

GB: Gall bladder, All figures are in percentage.

postoperative period as $P = 0.094$.

Therefore, similar acute phase responses are observed intraoperatively in patients fit for LC, when operated on by the open method with easy visceral tissue dissection. The Society of American Gastrointestinal and Endoscopic Surgeons (SAGES)[11] Guidelines assume greater significance in the light of the present study as better surgical skills will minimize the visceral tissue dissectional trauma with attenuation of the various acute phase responses that are the deciding factors for the final outcome as evident by per-operative modulation of TNF-α and C-reactive proteins. However, this also reflects that these selective stress markers (CRP and TNF-α) have a limited role in the clinical assessment of immune stress responses and tissue trauma. This finding warrants the search for more sensitive biomarker(s) for peri- and postoperative management of surgical patients.

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