SUMMARY OF THE THESIS

Hormonal, metabolic and immunological responses are the hallmark of surgical stress. Catecholamines, cortisol and glucose are important hormonal and metabolic biomarkers used to assess the extent of peri-operative stress responses. C-reactive proteins (CRP) and tumor necrosis factor-α (TNF-α) are specific proteins modulated by the acute stress of surgery. Injury to human body alters normal physiology across several systems, whether these alterations are proportional to the extent of injury at the individual level is still an issue of research and discussion.


The earliest consequence of a surgical incision is the rise in the circulating level of cortisol and other neurotransmitters (Wilmore et al, 1977 & 1980; Dubois et al, 1982). The circulating neurotransmitters remain high at two to five times for approximately 24 hours after major surgery (Watters et al, 1990; Marana et al, 1996). Initially the simultaneous secretion of the peptides as β-endorphin and adreno-corticotrophic hormone (ACTH) from the pituitary in response to acute stress was described in the rat (Guillemin and colleagues, 1977) and later on, nor-epinephrine hormone was considered as an important neurotransmitter in man (Silverberg et al, 1978).

Silverberg et al (1978) confirmed that onset of surgery is associated with prompt increase in plasma concentrations of noradrenaline & adrenaline and that this sympato-adrenal activation persists into the post-operative (post-op) period. Since then, an increase in circulating catecholamine and cortisol has been described in a variety of clinical conditions during anaesthesia and surgery including biliary tract surgery.
Biochemical markers such as ACTH, catecholamine, cortisol, glucagon, etc., all play crucial roles in the mediation of acute stress response. Catecholamines, cortisol and glucagon are released in high concentrations while insulin secretion decreases in response to trauma and sepsis. These changes are, in general, proportionate to the severity of the trauma and sepsis.

Recently alterations have been found to occur in every component of immune response during anaesthesia and surgery in a fashion parallel to the neurohumoral responses. These alterations represent the body's general physiological responses and are mainly dependent on the extent of surgery; however, other factors such as the patient's age, health status, medications, blood transfusion, etc. do play an important role in modulation of the acute stress response. Anaesthetic and operative complications have profound effects on these responses (Salo, 1992).

Upper abdominal surgery is usually associated with greater stress response as compared to the lower abdominal surgery that may prove more detrimental to the patient in terms of the systemic cardiovascular effects. In particular, tractions and manipulations during biliary tract surgery may lead to lethal consequences due to relative or absolute increase in sympathetic tone during cholecystectomy (Katz et al, 1970; Giesecke et al, 1988; Desborough, 1989; Haleem et al, 1991). Cardiovascular effects may include tachycardia, hypertension, dysrrhythmias, ventricular ectopics, angina or myocardial infarction & even pulmonary oedema during the intra-operative (intra-op) period and are important causes of the post-op morbidity & mortality. The pathway of above-mentioned phenomenon may either be: (a) through fifth thoracic segment of spinal cord, a common source of sympathetic supply to heart and gallbladder via cardiac plexus & celiac plexus respectively, (b) possibly mediated through reflex stimulation of adrenal gland via celiac plexus that get stimulated by surgical manipulations (Mendelssohn and Monheit, 1956; McGregor and DuPlessis, 1969; Warwick and Williams, 1973; Orloff, 1981).

Therefore, a change in anaesthetic and surgical technique is often made by experimental and clinical investigators in order to obtund the diverse hormonal metabolic changes to minimize peri-operative morbidity and mortality but resultant data have not been found consistently reproducible with respect to the desired modulation of the systemic stress response. Henrik Kehlet (1982) used extensive
epidural (up to T 4) anaesthesia to modify the surgery-induced adrenocortical responses, but he observed that the adrenocortical response could not be abolished completely during the upper abdominal surgery. Others have tried attenuation of stress response by intercostal and paravertebral blocks but with partial success. However, regional anaesthesia was recorded highly effective for attenuation of the surgical stress during pelvic and lower limb surgery. Modifications in general anaesthesia technique are often found not fruitful in this regard and even high dose opiates are not found fully efficient in early post-op period to attenuate the surgical stress (Klingstedt et al, 1987).

In addition to the hormonal metabolic response, a series of acute-phase reactants have been discovered during the surgical stress and has been extensively studied in recent years (Haq et al, 2004). Cytokines released from the injured and recruited cells are central to produce circulating factors including acute phase proteins for regulation of the immuno-inflammatory responses associated with trauma and infection.

Basically, the immune response to anaesthesia and surgery is a beneficial reaction, needed in local host defences and wound healing and in preventing the body from making auto-antibodies against its own tissues. These responses may contribute to the development of post-op infections and spread of malignant disease. During uncomplicated conventional surgery, the immune response usually passes clinically unnoticed without any harmful effects. Absent responses and excessively high responses, on the other hand, harm the patient (Salo, 1992).

Therefore in an attempt to attenuate the harmful effect of stress response to surgery the surgeons tried to evolve a technique which will do surgical resection without much intervention and tissue damage, thereby leading to the evolution of minimal access surgery into clinical practice. Initially attenuated neuroendocrine stress response after minimally invasive surgery was observed in pigs (Mansour et al, 1992). Later on, it was pointed out that the laparoscopic approach reduces the endocrine response to elective cholecystectomy (Schauer and Sirinek, 1995). The effect of gasless mechanical and conventional carbon dioxide pneumoperitoneum methods for laparoscopic cholecystectomy was also studied (Koivusalo et al, 1998). An attenuated acute phase response by minimal access surgery for cholelithiasis was observed by a number of workers (Glaser et al, 1995; Bruce and Smith, 1999). Comparative
evaluation of the various stress mediators in patients undergoing laparoscopic versus conventional cholecystectomy were extensively carried out in the recent past (Chaudhary et al, 1999; Sendt et al, 1999 Emirer et al, 2000; Demirer et al., 2000).

Local inflammatory peritoneal response to operative trauma in terms of certain cellular adhesion molecules (CAMs), different cell activity and cytokine release, etc., received focussed attention in the recent years.

Strenuous efforts have been made to compare post-op pain, pulmonary function, return of bowel function, energy metabolism, the resting energy expenditure (REE), etc., following open and laparoscopic cholecystectomy (Hendolin et al, 2000; Wilmore, 2002; Luo et al, 2003).

However, endoscopic surgery, mostly studied during laparoscopy, when compared with similar open operation, has, against all expectations, no important effects on classic endocrine metabolic responses but may slightly reduce inflammatory responses and various immune functions, although the data are not consistent (Kehlet, 1999).

Thus, in view of variable reports on hormonal, metabolic and immune responses following surgery, the present study entitled “A prospective biochemical study of systemic stress responses during cholecystectomy & abdominal hysterectomy” was planned and prospectively conducted, as the cholecystectomy and hysterectomy are the two most commonly performed procedures in our institution.

The current study measured and compared the magnitude of peri-operative stress in terms of six known stress biomarkers (Glucose, cortisol, epinephrine, norepinephrine, C-reactive protein and Tumor Necrosis Factor- alpha) in patients who underwent biliary surgery (laparoscopic cholecystectomy(LC) and conventional cholecystectomy (OC) and non-biliary lower abdominal surgery (open abdominal hysterectomy (OH). The study design also included assessment of the haemodynamic response in terms of heart rate (HR), systolic blood pressure (S.B.P.), diastolic blood pressure (D.B.P.) and mean arterial pressure (M.A.P.). Power of analysis warranted inclusion of 21 patients in each of the three study groups to have a 95 % confidence interval of difference (C.I.D.) with significance of p-value at 0.05.
Study protocol was approved by the Board of Studies (BOS) and Committee for Advanced Scientific Research (CASR) of our University.

Patients with symptomatic uncomplicated cholelithiasis fit for procedure were considered for cholecystectomy. Patients with acute cholecystitis, pancreatitis, choledocholithiasis, malignancy and jaundice, as well as patients with history of allergy, steroid intake, cytotoxic chemotherapy or hypoproteinaemia were excluded from the present study. Pregnant or lactating females who underwent cholecystectomy were not included in the study. Patients incurring intra-operative adjacent organ/structure injury or developing post-operative complications or requiring blood transfusion were also excluded from the study.

Open abdominal hysterectomy group considered only those patients who underwent total hysterectomy by open abdominal approach for benign uterine diseases. Patients with pan-hysterectomy done for carcinoma cervix, uterine malignancy, or patients on cytotoxic drug were excluded from the present study.

The patients were consented and discussed for specific procedures before surgery in the present prospective randomized study. Option of the surgical technique (laparoscopic or open) for cholecystectomy depended on many factors as existing financial circumstances of the patients (LC is 40% more expensive than open procedure (Barkun et al, 1995), patients’ preference for specific general surgeon who lack suitable expertise in laparoscopic technique, lack of facilities de novo/absence or malfunctioning of existing gazette as our institution is a government hospital, equipment failure do occurs in the form of lack of CO₂ cylinder supply or broken endoscopic instrument, patients’ insistence: patient’s phobia of complications after laparoscopic cholecystectomy based on bad experience of his/her kith & kin or laparoscopic surgeon were not available. These who were found fit for laparoscopic cholecystectomy on the pre-operative (pre-op) assessment were recruited for open cholecystectomy on the basis of non-medical reasons enumerated above.

Thus the open cholecystectomy group in the present study was a suitable control group for the laparoscopic cholecystectomy group in terms of the internal visceral tissue dissection.
In every patient, five blood samples (Pre-operative, ½ hour after start of surgery, end of surgery, post-operative days 1 & 4) were timely collected and analysed for the estimation of the serum concentrations of glucose, cortisol, epinephrine, norepinephrine, C-reactive protein and Tumor Necrosis Factor- alpha. All the same, haemodynamic clinical parameters were also religiously recorded at same five time points. It was thought prudent after-wise to calculate and analyse the rate-pressure product (RPP), a major determinant of myocardial oxygen consumption, as part of haemodynamic assessment although this was not included in the original study design.

Therefore, the present study included a total of 63 female patients who underwent cholecystectomy (open and laparoscopic) and open abdominal hysterectomy in the Jawaharlal Nehru Medical College Hospital, A. M. U, Aligarh (UP), India, and the study was completed over a period of 4 years from October 2004 to September 2008. All patients were operated upon under identical circumstances including anaesthetic-analgesic protocol and fluid regime (intraoperative as well as perioperative) after fully informed written consent.

The biochemical and clinical data were analyzed with standard computer software (SPSS version 17.0). Intra-group and inter-group comparisons were done by paired sample statistics and independent sample T tests respectively and comparison among the three groups was carried out with the help of ANOVA/ Bonferroni test. The p-value less than 0.05 was considered for the two-tailed significance. Correlation between two set of data were analysed with Pearson correlation and values between +0.7 to +1.0 were considered as strong positive associations.

I. Demographic Profile

The baseline characteristics of all patients were compared in term of age, weight, height, body mass index (BMI) duration of surgery and duration of anaesthesia and ASA grading.

1. Age

The Mean age ±SEM (Range) of the patients in the present study was 31.2±1.0 (18-42), 33.1±1.1 (22-50) and 44.9±1.1 (35-55) years in the LC, OC and OH groups
respectively (Table4-1). The age of the patients was comparable between both LC and OC groups (p>0.05), however, it differed significantly between LC vs. OH group and OC vs. OH group.

In developing countries like India, a sort of Western style epidemic of cholelithiasis is being increasingly observed and majority of these patients are in their 30s. This phenomenon is also seen in the present study. Ortega et al (1996) also reported almost similar age (Mean±SEM) of 34±2 and 33±3 years for LC and OC groups respectively. However, some other studies have reported higher age of patients undergoing cholecystectomy (Martin et al, 1992; Jatzko et al, 1995; Helmy et al, 1999; Hendolin et al, 2000; Sheen-Chen et al, 2002; Luo et al, 2003).

The age of the patients (Mean ±SEM) in the open abdominal hysterectomy (OH) group of the present study was expectedly higher than the age of patients undergoing cholecystectomy (p<0.001) as hysterectomy is usually indicated for benign diseases in majority of patients beyond the reproductive age or in the peri-menopausal age.

Moore et al (1994) reported patients’ age (Mean and Range) of 41.9 (36-45) and 43.3 (39-47) years for GA group (General Anaesthesia) and EDB group (GA + Extra-Dural Block) for total abdominal hysterectomy for benign disease. This is in full agreement with the finding of the present study (45±1.10 years; range 35 - 55).

**Age Range:** 18–42 years in the LC group; 22–50 years in the OC group; 35–55 years in the OH group. Martin et al (1992), Westerband et al (1992) and Joris et al (1993) reported an age range of 18 - 91 years, 22 – 82 years and 20 – 69 years respectively for patients undergoing laparoscopic cholecystectomy.

2. **Sex**

All patients in the present study belonged to the female sex not only in the open abdominal hysterectomy group but also in the laparoscopic and open cholecystectomy groups, eliminating the gender bias in the present study, especially with respect to comparison between uterine surgery (hysterectomy) and biliary surgery (cholecystectomy). Furthermore, this also eliminates the recently recognized differences in the stress responses between males and females.
All of them were neither pregnant nor lactating at the time of cholecystectomy in both laparoscopic and open groups. None of them was menstruating at the time of operation.

3. Weight

Patients' weight (Mean ±SEM) was 45.0±1.0 kg in LC group (range 40-56), 46.5±1.1 kg in OC group (range 40-60) and 52.3±1.3 kg in OH group (range 43-62).

The weight was found comparable between LC and OC groups (p>0.05) while it was found significantly different between LC vs. OH group (p<0.001) and OC vs. OH group (p<0.05).

4. Height

Patients in LC, OC and OH groups had the Mean height ±SEM (range) of 155.4±1.0 (150-162), 155.8±0.8 (150-163) and 156.7±0.7 (152-164) cms respectively.

The height was found comparable between LC vs. OC groups and LC vs. OH groups but significant difference was noted between OC vs. OH groups on t-test analysis. However ANOVA and Bonferroni tests verified that there was statistically no significant difference among the three groups - p=0.792 between LC vs. OH, p=1.0 between LC vs. OC and p=1.0 between OC vs. OH.

5. Body Mass Index (BMI)

All patients in the present study had normal weight as per the WHO criteria (<25 Kg.m\(^{2}\)) and BMI was found 18.60, 19.12 and 21.26 in the LC, OC and OH groups respectively. Overweight/obese patients were not included in the present study. The BMI was comparable between LC and OC groups (p<0.05). However, it differed significantly between LC vs. OH groups and OC vs. OH groups (p<0.001).

In 1993, Joris and associates from Belgium reported patients’ mean weight of 66.8 kg and mean height of 1.65 meter for patients undergoing lap chole, resulting in the BMI of 24.5, that is, less than 25 Kg.m\(^{2}\), the normal BMI as per the WHO criteria. This is in full agreement with our own finding.

Chambrier and associates (1996) reported BMI (Mean±SEM) of 23.1±1.3 and 24.3±1.2 in two groups (Placebo & Ibuprofen) of patients who underwent open cholecystectomy at Lyon, France, that is, their patients’ BMI was <25 Kg.m\(^{2}\) as was
seen in the present study. Recently Luo et al (2003) also reported from China a BMI of 24.8 and 23.5 Kg.m$^{-2}$ in LC and OC groups respectively with insignificant intergroup difference (p>0.05).

However, in recent past, some investigators reported BMI for patients undergoing cholecystectomy a little higher than that recorded in the present study (Martin et al. 1992; Rademaker et al, 1992; Hendolin et al, 2000). Glaser et al (1995) reported a BMI of 26.7 and 25.8 Kg.m$^{-2}$ in LC and OC groups respectively with insignificant intergroup difference (p>0.05).

Thus, We find that mean age and BMI of the patients in the present study do not conform to the usual notion as described by Rains and Ritchie (1982) who reported gallstones in only 20 % of women during child-bearing age and in fatty females. This is in agreement with the study of Ortega et al (1996) and suggests a definite change in the incidence of cholelithiasis with respect to not only to the age but also the body mass index.

*The present study recorded following changes in the hormonal, metabolic, acute-phase reactant and immunological markers during the acute stress state following surgery in the three study groups (LC, OC & OH).*

**II. Metabolic Responses**

In term of serum glucose, an accelerated metabolic response was noted during perioperative period in the three groups. In LC group, the pre-op blood glucose value (96.71 mg/dl) increased to 154.86 mg/dl (60.13 %↑) after half an hour after start of surgery and 156.29 mg/dl (61.61 %↑) at end of surgery which was highly significant (p<0.001). However, in the post-op period on 1$^{st}$ and 4$^{th}$ post-op days, glucose concentrations were recorded 98.57± 1.92 mg/dl (1.92 %↑) and 97.62± 2.229 mg/dl (0.94 %↑) respectively, and both these values were not significantly different from the pre-op value (p=0.448 & p=0.623 respectively).

In OC group, pre-op value of blood glucose (96.38± 2.1903mg/dl) level increased to 144.05±0 3.22 mg/dl (49.46% ↑) at half-an-hour after start of surgery and148.62±2.87 mg/dl (54.20% ↑) at the end of surgery, and the increase in both these values proved significant (p<0.001). On 1$^{st}$ and 4$^{th}$ post-op days, glucose concentrations were
recorded 124.38± 1.01 mg/dl (29.05 %↑) and 104.90±2.03 mg/dl (8.84 %↑) respectively, and both these values were significantly higher than the pre-op baseline value (p<0.001).

In OH group, pre-op baseline value of blood glucose (96.4286± 2.30 mg/dl) increased to 138.143± 1.39 mg/dl (43.26 %↑) at half-an-hour after start of surgery and 149.71±1.74 mg/dl (55.25% ↑) at the end of surgery, these observed values were statistically significant (p<0.001). On 1st and 4th post-op days, glucose concentrations were recorded 121.952±1.717 mg/dl (26.27 %↑) and 108.1429± 1.63 mg/dl (12.14% ↑) respectively, and these values were significantly higher than the pre-op baseline value. Thus in the open cholecystectomy and open hysterectomy groups, the blood glucose level did not return to the baseline pre-op value even on the 4th post-operative day.

Comparable baseline pre-op blood glucose levels in the laparoscopic and open cholecystectomy & open hysterectomy groups was evident by ANOVA/ Bonferroni test (p= 0.995), indicating that the three groups are really comparable in terms of the metabolic response.

The changes following surgery were statistically significant (p<0.001) from each other at half-an-hour after start of surgery between laparoscopic cholecystectomy and open abdominal hysterectomy, although they were insignificant (p=0.263) between open cholecystectomy and open hysterectomy.

At completion of surgery, comparable rise in blood glucose level was noted between laparoscopic cholecystectomy and open hysterectomy (p=0.131) as well as between open cholecystectomy and open hysterectomy (p=0.935). Multiple comparisons by Bonferroni test verified the changes in laparoscopic cholecystectomy and open hysterectomy groups.

In the present study, rapid accelerated metabolic response with high plateau during intra-operative period in LC group points towards additional strain of pneumoperitoneum with peritoneal stretching as also emphasized by Rademaker et al (1994). This is indirectly supported by the early normalization of glycemic response on the very 1st postop day.
Thus, in the light of earlier reports (Engquist et al, 1977; Tsuji et al, 1983 and Moore et al, 1994) on the inhibitory effect of epidural block on surgery-induced hyperglycaemic response, direct nervous system stimulation secondary to intra-abdominal visceral traction and stretching during open surgery appears to be an obvious plausible cause for enhanced secretion of cortisol and catecholamines which is known to facilitate glucose production and which is recorded high in the present study and hence the intra-operative hyperglycaemic response in the open groups is seen in the present study. Increased myocardial oxygen consumption after surgery may be another explanation for extended metabolic responses following open surgery. This is in accordance with our own observation of increased myocardial oxygen consumption as > 20 % increase in rate-pressure product (RPP) during open cholecystectomy and 14 % following open abdominal hysterectomy recorded in the present study. The adverse influence of wound pain on respiration with resultant hypoxaemia cannot be ignored as it is known to produce heightened glycemic response (Chumillas et al, 1998; Ali and Gana, 1998; and Larsen et al, 2002) and it has more pronounced and long-lasting impact on patients’ convalescence in open cholecystectomy than in laparoscopic surgery. This may be the reason why the intergroup difference in the glycemic response was found significant (<0.001) on the 1st and 4th post-op day between both the lap and open groups and insignificant (>0.05) between the two open groups (OC vs. OH).

III. Hormonal Responses

1. Cortisol response

Significantly altered stress responses were also evident in the expression of Serum Cortisol. In LC group, pre-op level (423.00±25.96 nmol/L) increased to 589.33±24.62 nmol/L (39.32% ↑) at half-an-hour after start of surgery, and 710.86±30.14 nmol/L (68.05 % ↑) at the end of surgery (p<0.001). On post-op day 1, cortisol level was recorded 439.48±22.76 nmol/L (3.90 %↑; p=0.573) and on post-op day 4, it was recorded 311.24±58.51 nmol/L (26.42 % ↓; p=0.001).

However, in OC group, pre-op cortisol level (341.476±20.86) increased significantly by 84.48 % and 128 % (p<0.001) at half-an-hour after start of surgery and at the end
of open surgery respectively. The high cortisol levels persisted on the 1\textsuperscript{st} post-op day (74.42 %↑) and 4\textsuperscript{th} post-op day (44.47 %↑) (p<0.001).

In OH group, the baseline value of serum cortisol (325.38±10.2951nmol/litre) increased to 102.16 % and 184 % at half an hour after start of surgery and at end of surgery respectively. In the postop period the level was still raised to 92.77 % and 67.305 % above the baseline at 1\textsuperscript{st} and 4\textsuperscript{th} post-op day, in a fashion similar to open cholecystectomy. Bonferroni test for multiple comparisons among groups was significant at the end of surgery and in the postop days 1 & 4. The p values were found <0.05 between LC vs. OH groups and <0.001 between OC vs. OH groups at the end of surgery.

On the first postop day, the values were significantly different between LC vs. OC groups (p<0.05) and LC vs. OH groups (p<0.001), but the intergroup difference was insignificant between OC and OH groups (p=1.00). On 4\textsuperscript{th} post-op day, the intergroup differences again proved significant between LC vs. OC groups and LC vs. OH groups (p<0.001), and insignificant between OC and OH groups (p>0.05).

The short-lived accelerated cortisol response during the intra-operative period in the LC group with rapid normalization to the pre-op level on the very 1\textsuperscript{st} post-op day is in full agreement with the observations of other investigators (Joris et al, 1992; Mealy et al, 1992; Redmond et al, 1994, Ortega et al, 1996; Aono et al, 1998; Hendolin et al, 2000; Mendoza-Sagaon et al, 2000; Crema et al, 2005).

The peritoneal stretching during laparoscopy seems to be the important contributory factor for accentuated hormonal (and metabolic) response with stimuli for the stress response arising from visceral and peritoneal afferent nerve fibres in addition to those from the abdominal wall as emphasized by Rademaker and colleagues (1994a), Desborough (2000) and Adhikary & Korula (2004).

2. Catecholamine Response

In response to surgical stress, the levels of Catecholamines (epinephrine and norepinephrine) altered markedly in all the three groups. Percentage increase in pre-op serum epinephrine level at half an hour after incision was 85.47 % (LC group), 141.44 % (OC group) and 102.70 % (OH group). At the end of surgery serum epinephrine concentrations increased by 189 %, 258 % and 251 % in the LC, OC
and OH groups respectively. However, the differences among groups on application of Bonferroni test were not significant from each other during intraoperative period, including half an hour after incision and end of surgery (p>0.05).

The accelerated serum epinephrine responses continued in the post-op period in both the conventional groups (open cholecystectomy and open hysterectomy) and epinephrine level returned towards pre-op levels in the laparoscopic group (28%, 173 %, and 164 % increase above the pre-op level in LC, OC and OH groups respectively; p<0.001). On 4th post-op day, epinephrine responses were comparable between both the conventional groups (p>0.05) but differed significantly between LC and OC groups, which was confirmed by ANOVA/ Bonferroni test.

In the LC group of the present study, there was slow intraoperative rise in epinephrine concentration with peaking at the end of surgery (approximately 3-fold increase) and then it decreased markedly on the 1st post-op day though still different statistically from the pre-op value (<0.05). This observation is in full agreement with the observations of Ratge et al (1990), Anand and Hickey (1992), Glaser et al, (1995) and Ortega et al (1996).

Thus, LC was associated with marked intra-operative systemic stress response which was continued in the post-operative period until 1st post-op day. On 4th post-op day in the LC group, the epinephrine concentration decreased further and was recorded 17 % lower than the pre-op value, indicating presence of pre-op stress in these patients most probably secondary to the procedure-related anxiety and apprehension, mainly in relation to newer technology as seen also in relation to the cortisol response in the present study. In other words, pre-op concentration is often not reflective normal basal value of the catecholamines, and this observation should be kept in while interpreting the data.

Norepinephrine (NE) levels augmented considerably at half an hour after and at the end of surgery. The changes follow the similar pattern as epinephrine. NE level increased by 116% (LC), 108 % (OC) and 88% (OH) at the end of surgery. Intergroup differences were not statistically significant (p>0.05) from each other during intra-operative period (ANOVA significance =0.211 and 0.120, at half hour after incision and end of surgery respectively). However, at the first post-op day significant differences were noted among the three groups (p < 0.001). ANOVA/
Bonferroni test revealed that the changes were significant between LC vs. OC groups and LC vs. OH groups, but insignificant (p>0.05) between OC and OH groups. However, at 4\textsuperscript{th} postop day norepinephrine responses became similar among groups. The changes in hormonal markers were more intense in hypertensive patients as compared to normotensive ones (p<0.05).

The degree of association between the two biomarkers (epinephrine and norepinephrine) was found strongly positive on application of Pearson correlation (LC group Pearson correlation=0.995 and p<0.001; OC group Pearson correlation=0.979 and p=0.004 and OH group Pearson correlation=0.982 and p=0.003).

**IV. Acute-Phase Reactant Response**

Present study recorded altered expression of **C-reactive protein** (CRP) following surgical intervention. CRP level did not increase in serum from the baseline value half an after start of surgery (p=0.081) in LC and OC groups, while increased markedly (p<0.001) in hysterectomy group.

Significant increase in CRP concentration was noted at the end of surgery as well as post-operative period till day 4\textsuperscript{th} (p value < 0.001). The inter-group difference was not significant among groups till end of surgery (p value > 0.05).

In all the groups, the increased serum CRP concentration reached the maximum level at 24-48 hours after surgery and started declining gradually from 4\textsuperscript{th} day of surgery. However, significant difference between laparoscopic cholecystectomy and open hysterectomy was noted at 1\textsuperscript{st} postop day (p<0.001).

The degree of CRP enhancement was noted >6 folds after open surgery, and > 3 folds following laparoscopic surgery.

**V. Immunological Response**

TNF expression in the LC group did not change till 1\textsuperscript{st} post-op day and its expression doubled on the 4\textsuperscript{th} post-op day but this was found statistically insignificant (p>0.05).

This observation of the delayed insignificant TNF-alpha expression in the late post-op period confirms the opinion that laparoscopic intervention preserves the peri-
operative immune responses as has also been reported recently by a number of investigators (Redmond et al, 1994; Allendorf et al, 1996; Berguer et al, 1998; Walker et al, 1999; Siestses et al, 2000; Sheen-Chen et al, 2002).

In the OC group, TNF-alpha concentrations did not increase in the intra-operative period. TNF was expressed many folds on the 1st & 4th post-op days but these expressions were statistically insignificant (p>0.05).

On the 4th post-op day, one out of 21 patients developed excessively high TNF enhancement as compared to other patients in this group but this did not alter the results of the group, validating the adequacy of the group sample size calculated by the power of analysis.

In the OH group, TNF-alpha concentrations did not increase at all in the intra-operative period. Significant enhancement of TNF-alpha expressions (p<0.001) was seen on the 1st post-op day which increased further on the 4th post-op day (p<0.001).

In both the LC and OC groups, TNF expression showed a characteristic pattern of delayed insignificant rise in the late post-op period and the intergroup difference was also found insignificant (p>0.05), indicating similar degrees of immune preservation following open cholecystectomy in patients fit for laparoscopic cholecystectomy as well as following laparoscopic cholecystectomy. This means that internal visceral tissue dissectional trauma really determines the immunologic modulations and difference in the amount of the parietal tissue dissectional injury (2 cms in LC and 6-9 cms in OC) does not really matters as far as immune function is concerned.

It is of interest to note that highly significant post-operative enhancement of TNF-alpha expressions in the OH group (p<0.001) does not differ from the insignificant post-op TNF expressions in LC & OC groups (p>0.05) on statistical analysis by ANOVA/ Bonferroni test. This is difficult to explain and may need better computational measures or larger sample size to fully elucidate the difference.

Attempt was made to correlate the expressions of TNF-alpha and CRP but the significant increases in CRP level following surgery were not correlated with the TNF-α expressions following surgery in the LC group on application of bivariate correlation by Pearson test (r=0.279; p> 0.05).
However, there was found strong correlation associations in TNF and CRP expressions in the both the OC and OH groups (r= 0.765 and r=0.821 respectively) but these correlations were found statistically insignificant (p>0.05) in both the open groups. This emphasizes the point that sometimes Pearson correlation significance and p-value significance may differ and caution is required while interpreting the data.

Parallel changes in the acute-phase reactant and immune responses in the two open groups secondary to a certain amount of the surgical trauma. This support the opinion of Ohzato et al (1992) that TNF-alpha is a possible hepatocyte-stimulating factor (HSF) for inducing production of acute-phase proteins by the hepatocytes. They reported that ΔCRP was closely correlated (r = 0.623, p<0.001) with Δ Cytokine (IL-6) in patients who underwent upper abdominal open surgery including cholecystectomy (n=3).

VI. Haemodynamic Responses

Heart Rate (HR), Systolic Blood Pressure (SBP), Diastolic blood Pressure (DBP) and Mean Arterial Pressure (MAP) were noted following every procedure and analysed in three groups/subgroups separately including normotensives and treated hypertensives.

1. Heart Rate

All the three groups showed almost similar pattern of significant tachycardia throughout the whole procedure in LC as well as OC group, and only in the later part of the procedure in the OH group. This may simply imply that the reflex stimulation of suprarenal nerve plexuses via the celiac ganglion may produce adrenal bursts and overall sympathetic over activity. Fifth thoracic segment of the spinal cord is a common source of sympathetic supply to the heart through cardiac plexuses (Warwick and Williams, 1973) and to the biliary tract through celiac plexus (Warwick and Williams, 1973; McGregor and Du-Plessis, 1969; Orloff, 1981). Warwick and Williams (1973) emphasized that the higher branches of the greater splanchnic nerves may be traced upwards in the sympathetic trunk as far as second or even first thoracic ganglia. Hence, stretch or distension sensations of the biliary tract, transmitted through visceral sensory fibres of the splanchnic nerves, may lead to relative or
absolute increase in the sympathetic tone, causing tachycardia, hypertension and even tachyarrhythmias (Orloff, 1981; Katz and Bigger, 1970).

The present intraoperative observations in the biliary surgery (both open and lap) are in full agreement with those of Mendelsohn and Monheit (1956), Desborough et al (1989) and Haleem et al (1991). This indirectly also supports the observations in the open hysterectomy group in the light of the anatomical distribution of sympathetic nerve supply.

In the post-operative period, all the three groups exhibited not only normalization but also decrease of the heart rate lower than the pre-operative level – the decrease being statistically significant (p<0.05) only in LC group and insignificant (>0.05) in OC & OH groups.

The most probable explanation for this observation may be that the patients were already under significant degree of stress even before induction of anaesthesia and surgery due to marked pre-operative anxiety and apprehension, mainly in relation to newer technology as has already been discussed in detail under the heads of cortisol and epinephrine. However, it appears prudent to again highlight the two important things- one, the need of thorough pre-operative counselling for anxiolysis, and second, the need of sampling at the time of PAC (Pre-Anaesthetic Check up) for baseline values or at least at the time of admission a day before operation. Sampling at the time of admission to the hospital may again be a cause for concern with respect to the fast evolving Day Case Surgery or Fast Track Surgery.

2. Arterial Blood Pressures

In the **LC group** of the present study, all variables of arterial serum pressure (Systolic, diastolic and mean) changed in a manner similar to HR and showed significant intra-operative increase with rapid peak effect at half-an-hour after incision, and significant post-operative decrease which was found lower that the pre-operative level even on the 4\textsuperscript{th} day (p<0.05).

Similar to the current study, accelerated cardiovascular changes during laparoscopic surgery were reported by a number of clinical and experimental investigators (Johannsen et al, 1989; Torrielli et al, 1990; Westerband et al, 1992; Rademaker et al, 1994; Dorsay et al, 1995; Bannenberg et al, 1997; Branche et al, 1998).
In the **OC group**, all variables of arterial serum pressure (Systolic, diastolic and mean) were increased significantly during intra-operative period with minor differences at half-an-hour and end of surgery, and all of them returned to near or even below the pre-operative level on the very 1st postop day.

These observations are at a little variance to those seen in the LC group where rapid peaking effect at half-an-hour was noted in all variables of arterial serum pressure (Systolic, diastolic and mean).

This implies that both open and lap chole procedures are equally stressful with similar intra-cavitatory management with only one difference at half-an-hour of incision and that is the additional mechanical effect of the pneumoperitoneum during lap chole which was not found to be additive in the subsequent part of the procedure.

In the **OH group**, there were significant intra-operative increases in all variables of arterial serum pressure (Systolic, diastolic and mean) which were followed by normalization on the very 1st post-operative day. However, it is interesting to note that systolic and mean arterial serum pressure achieved peak at half-an-hour after incision while heart rate and RPP showed peaking effect at the end of the surgery.

**Comparison among LC, OC & OH groups:** The time course pattern was found similar in systolic, diastolic and mean arterial serum pressures among the three groups. However, arterial blood pressure in all denominations was more pronounced in the intra-operative phase of the laparoscopic cholecystectomy in the present study. The possible mechanisms responsible for this accentuation phenomenon during laparoscopic cholecystectomy include mechanical compression & stretching effects of pressure pneumoperitoneum and release of humoral substances secondary to the pneumoperitoneum and surgical tissue dissectional injury. Carbopneumoperitoneum-induced hypercarbia was not considered as a plausible in the present study aetiological factor in the present study due to the religiously maintained ETCO$_2$ level within a range of 32 to 35 mmHg.

### 3. Rate-Pressure Product

Rate Pressure Product (RPP), product of heart rate and systolic blood pressure, is a major determinant of myocardial oxygen consumption.

It increased during intra-operative period in all the three groups and decreased markedly to lower than the pre-op value. In LC group (combined subgroups including
normotensives and hypertensive subgroups) during intraoperative period maximum increase in RPP value was noted at half an hour after start of surgery with rapid peaking (29.6 %). These changes declined to lower than the pre-op value (8.5-9.5 %) in the post-operative period.

The OC (combined) group also revealed persistently high RPP (21 to 23 % increase) value in a lesser magnitude to LC, throughout the procedure and then post-operative normalization to the pre-op value (<3 % difference). However, OH (combined) group showed gradual intra-operative rise in RPP with a maximum of 14 % increase at the end of surgery and then post-op decrease to lower than the pre-op value by 4 to 5.5 %.

The present study noted greater intra-operative increase in RPP in hypertensive subgroup as compared to normotensive subgroup in all the three groups (LC, OC & OH). The maximum rise occurred at half an hour after incision and post-operative decrease to lower than the pre-op value by 4 to 9.5 %.

The normotensive subgroup showed intra-operative increase in RPP in a similar manner to combined subgroup but to a lesser degree and post-operative decrease to lower than the pre-op value by 8.6 to 9.5 % in LC group and complete normalization to the pre-op value in OC group.

However, OH-normotensive subgroup showed minimal rise (<2 %) at half an hour after incision and mild increase at the end of surgery (11 %) and then post-operative decrease to lower than the pre-op value by 7 %.

Critical changes in RPP was observed which crossed the upper limit of RPP to the dangerously high level in the hypertensive patients undergoing laparoscopic cholecystectomy soon after start of surgery (half an hour), suggesting real high risk in these patients.

However, intraoperative changes in RPP persisted to some extent (8 %) on post-op day 1 in hypertensive patients of LC group in absence of complication, and this indicates continued higher myocardial oxygen consumption even after 24 hours of surgery that is apparently correlated to the high pressure pneumoperitoneum. Thus insult associated with pneumoperitoneum cannot be under scored especially in high risk cardiopulmonary disease patients (Wittgen et al, 1991).
Presence of significant degree of pre-op mental stress related to surgery-induced anxiety and apprehension, especially with respect to the newer technology was evident with fall of RPP 4 to 9.5 % below the pre-op value on the 4th post-op day in all subgroups except OC group (normotensive). Thus role of pre-op anxiety in augmenting the surgical stress response cannot be ignored in the era of fast track surgery.

Search of the available literature reveals that thrust on the measurements of metabolic hormonal changes and immune expressions has taken precedence over the measurements of basic clinical parameters of the cardio-vascular changes that are continuously monitored during any surgical procedure, more so during the laparoscopic procedure in developing countries with limited resources and limited public access to specialized centres.

VII. Correlations Between Different Systemic Responses

1. Correlations between Metabolic and Hormonal Responses

In the present study, strong positive correlations (Pearson, r >0.7) were found between serum glucose and serum cortisol concentrations and between the serum glucose and serum catecholamines (both epinephrine and norepinephrine) concentrations over the longitudinal course of the study in all the three groups. These findings of significant metabolic and hormonal correlations in the present study are in full agreement with the observations of Glaser et al (1995).

2. Correlations among Hormonal Responses

Strong positive correlations (Pearson, r > 0.7) were also observed between serum cortisol and serum catecholamines (Both epinephrine and norepinephrine) and between serum epinephrine and serum norepinephrine concentrations in all the three groups (LC, OC and OH) throughout the peri-operative period, suggesting parallel increases in the two catecholamines in response to a certain degree of the surgical trauma in laparoscopic as well as open groups of the present study.

3. Correlations between Haemodynamic and Hormonal Responses

Serum epinephrine and norepinephrine concentrations showed different correlations with the haemodynamic parameters in the laparoscopic and open groups over the longitudinal course of the study.
In the **LC group**, strong positive correlations (Pearson, $r > 0.7$) were found between serum norepinephrine concentrations and haemodynamic parameters (Heart rate, arterial blood pressures (systolic, diastolic and mean) and rate-pressure product) but the correlations between serum epinephrine concentrations and haemodynamic parameters (Heart rate, arterial blood pressures (systolic, diastolic and mean) and rate-pressure product) were found only weak positive (Pearson, $r < 0.7$).

Significant intra-operative increases in the arterial blood pressures (Systolic, diastolic and mean) observed in the present study were accompanied by the significant intra-operative increases in plasma epinephrine and norepinephrine concentrations in the present study. It is to be noted that intra-operative increase in arterial blood pressures and plasma concentrations were individually highly significant but the time course pattern was found different: peaking of the arterial blood pressures (Systolic, diastolic and mean) occurred at half-an-hour after incision while plasma catecholamines achieved peak at the end of surgery, suggesting interplay of additional factor(s). The most probable factors for maximal haemodynamic changes soon after creation of the pneumoperitoneum include pneumoperitoneum-induced compression of intra-abdominal great vessels as emphasized by majority of investigators or direct sympathetic nervous system stimulation as emphasized by Katz and Bigger (1970), Orloff (1981) and Haleem et al (1991) or increased sympathetic tone resulting from reflex activity (Marshall et al, 1972)/ baro-reflexes (Greim et al, 2003) or vasopressin release secondary to peritoneal stretching/ stimulation and decreased venous return to the heart as emphasized by Lentschener et al (2001).

In the **OC group**, the correlations of s-norepinephrine as well as s-epinephrine with almost all the haemodynamic parameters were found weak positive (Pearson, $r = +0.3$ to $+0.7$) except in the case of epinephrine vs. systolic blood pressure where there was found no correlation at all (Pearson, $r < 0.3$).

Therefore, the causal mechanisms for these changes may include peritoneal stretching and stimulations in open surgery causing vasopressin release as emphasized by Lentschener et al (2001), or direct stimulation of sympathetic nervous system as emphasized by Katz and Bigger (1970), Orloff (1981) and Haleem et al (1991), or increased sympathetic tone resulting from reflex activity (Marshall et al, 1972).
The present observations in the OH group is in partial agreement with that of Bickel et al (1991) who did not find any relevant correlation on linear regression analysis between catecholamines (epinephrine & norepinephrine) and haemodynamic changes even at a single time point during open cholecystectomy.

In OH group, correlations of arterial serum pressure changes and plasma catecholamine changes were found variable with different clinical parameters in a fashion similar to those seen in the OC group, the open surgery group. Hence, role of other factors involved in the open surgery assumes importance for causation of equally significant alterations in arterial blood pressure and other haemodynamic parameters (McKenzie et al, 1980; Kelman et al, 1972, Motew et al, 1973).

VIII. Correlations with Duration of Surgery

1. Metabolic Response and Duration of surgery
Correlations between the glucose response and the duration of surgery were found entirely different in the three groups – weak positive in LC group, strong negative in OC group and strong positive in the OH group, suggesting that the operating time has little or no significance with respect to the magnitude of the metabolic response under different techniques but the severity and the extent of the surgical tissue dissectional injury may play sole or bigger role for the degree of the surgical stress response as suggested by Kristiansson et al (1999).

2. Hormonal Responses and Duration of surgery
Cortisol’s correlation with the duration of surgery followed the pattern in all the three groups exactly similar to the glucose’s correlation to the duration of surgery, viz., weak positive in the LC group, weak negative in the OC group and strong positive in the OH group. In other words, correlations between the cortisol response and the duration of surgery were also different in the two open groups.

Strong positive correlations were observed between the duration of surgery and epinephrine response in the laparoscopic group and also in both the open groups ($r > +0.7$).
Correlations between the norepinephrine response and the duration of surgery was found only weak negative (r = -0.7 to -0.3) in the lap chole and open chole groups but strong positive correlation (r = +1.0) was detected in the open hysterectomy group.

3. Acute-Phase Reactant Response and Duration of surgery

All the three groups recorded strong positive correlations between the duration of surgery and CRP expressions (r > +0.7), suggestive of increasing inflammatory response with the increasing duration of surgery. However, Ohzato et al (1992) found no correlation between ΔCRP and duration of surgery (r = 0.307, NS) in patients who underwent upper abdominal open surgery including cholecystectomy (n=3).

4. Immune Response and Duration of surgery

Strong negative correlations were detected between TNF-alpha expression and duration of surgery not only in the LC group but also in the OH group. On the other hand, the OC group recorded no correlation between TNF-alpha expression and duration of surgery.

Kristiansson et al (1999) did not find any correlation between cytokine expression and duration of surgery in LC as well as OC group. Ohzato et al (1992) reported close correlation between Δ Cytokine (IL-6) and duration of surgery (r = 0.497, p<0.01) in patients who underwent upper abdominal open surgery, including cholecystectomy (n=3).

Our findings are in partial agreement with observations of Cruickshank et al (1990) who recorded positive correlation between duration of surgery and magnitude of cytokine expression (IL-6) in patients undergoing elective surgery of varying severity. These observations support the suggestion of Kristiansson and colleagues (1999) that the severity and the extent of the trauma (Surgical tissue dissectional trauma) are possibly more important than the operating time per se for the magnitude of surgical stress responses in terms of various mediators and biomarkers. This is becoming more evident in view of the fact that in recent times, the complex surgeries are increasingly carried out for hours together by both techniques – laparoscopic and open but without gross homeostatic disturbances and with favourable outcomes. However, their suggestion, based on only cytokine expressions, of lap chole to be regarded as a minor surgical procedure appears untenable because it is the net result of the
multifactorial interactions of a battery of homeostatic factors, some understood appreciably and many others still less or even least understood especially under clinical circumstances, that will decide the magnitude of a surgical operation so that therapeutic modulations and interventions may lead to the improved quality of patient care as well as to achieve the favourable outcome not only satisfactory to the surgico-anaesthetic team but also, more importantly in the current era of the expanding marketing forces, satisfactory to the increasingly aware patients.

To conclude, the present study demonstrated specific changes in the six studied stress biomarkers including serum glucose, cortisol, epinephrine, norepinephrine, CRP and TNF α levels as well as clinical monitored parameters following laparoscopic and open surgery. We found cholecystectomy and hysterectomy are stressful procedures which modify the stress response significantly, either performed through classical open method or laparoscopic technique. Following laparoscopic surgery, the stress hormonal responses were more pronounced during intraoperative period due to the additional mechanical effects of high pressure pneumoperitoneum although associated with earlier post-operative normalization and this procedure should be taken as seriously as any major surgery not only in the hypertensive patients but also in the normotensive patients. In the post-operative period, catabolic hormonal responses were more obvious and continued after open abdominal hysterectomy and open cholecystectomy.

In nutshell, the present work does verify the rapidly evolving current evidence that the laparoscopic cholecystectomy is as stressful as the open surgery intraoperatively in terms of metabolic, hormonal and haemodynamic responses. Furthermore, laparoscopic cholecystectomy patients experienced significant peri-operative immune preservation (Minimal TNF-alpha alterations in intra-and post-operative periods as compared to the open surgery groups) and much less acute-phase reactant expression (50 % CRP expression less than in open surgery groups). Thus, the present study confirms that the earlier normalization of the metabolic hormonal responses, lesser acute-phase reactant expressions and significant peri-operative immune preservation following laparoscopic cholecystectomy are possible major determinant factors that usually lead to the commonly observed distinct clinical efficacy of laparoscopic cholecystectomy.
REFERENCES


