Abstract

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.

Endocrine-metabolic response to surgery has been extensively studied since long albeit with varying results.

The earliest consequence of a surgical incision is the rise in the circulating levels of cortisol, noradrenaline & adrenaline which remain high at two to five times for approximately 24 hours after major surgery. ACTH, glucagon, etc., also play crucial roles in the mediation of acute stress response. Their 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. Furthermore, a series of acute-phase reactants have been discovered during the surgical stress and has been extensively studied in recent years. 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. Local inflammatory response to operative trauma in terms of certain cellular adhesion molecules (CAMs), different cell activity etc., received focussed attention in the recent times.

These alterations represent the body's general physiological responses and are mainly dependent on the nature and extent of surgery; however, other factors such as the patient's age, health status, medications, blood transfusion, anaesthetic technique, sepsis, etc. do play an important role in modulation of the acute stress response.

Anaesthetic and operative complications have profound effects on these responses.

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. Comparative evaluation of the various stress mediators in patients undergoing laparoscopic versus conventional cholecystectomy were extensively carried out in the recent past but with varying results.

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.

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.

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, 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).
Demographic Profile

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. 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.
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.
Age Range: 18–42 years in the LC group; 22–50 years in the OC group; 35–55 years in the OH group

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).

Thus, We find that mean age and BMI of the patients in the present study do not conform to the classical teaching that gallstone is a disease of fatty females above fifty, suggesting 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):

A. Metabolic Responses:

In term of serum glucose, an accelerated metabolic response was noted during peri-operative period in the three groups. Pre-op blood glucose levels were comparable among the laparoscopic cholecystectomy (LC), open cholecystectomy (OC) & open abdominal hysterectomy (OH) groups (p>0.05).

In LC group, the pre-op blood glucose value (96.71 mg/dl) increased significantly during intra-op period (p<0.001). However, glucose concentrations recorded in the post-op period were not significantly different from the pre-op value.
In the open groups (OC and OH), pre-op values of blood glucose (96.38± 2.1903 and 96.4286± 2.30 mg/dl respectively) increased significantly during intra-operative period (p<0.001). Furthermore, glucose concentrations recorded in the post-op period were also significantly higher (p<0.001) than the pre-op values (cf. LC group).

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.

The changes following surgery were significantly different (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, rise in blood glucose level was comparable between laparoscopic cholecystectomy and open hysterectomy (p=0.131) as well as between open cholecystectomy and open hysterectomy (p=0.935).

Thus, rapid normalization of the accelerated metabolic response on the very 1st postop day in the LC group points towards additional strain of temporary high pressure pneumoperitoneum with peritoneal stretching.

B. Hormonal Responses:

1. Cortisol response

In LC group, pre-op level (423.00±25.96 nmol/L) increased significantly (39.32% ↑) in the intra-op period (p<0.001). On post-op day 1, cortisol level decreased with insignificant difference from the pre-op value (3.90 %↑; p=0.573) and on post-op day 4, it decreased further and became 26.42 % lower than the pre-op level (p=0.001).

However, in both the open groups (OC and OH), pre-op cortisol levels (341.476±20.86 and 325.3810± 12.2951 nmol/litre respectively) increased significantly throughout the intra-operative period (p<0.001). The high cortisol levels persisted in the post-op period till 4th post-op day (p<0.001).

Intergroup differences among the three groups were 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 4th 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 1st post-op day is, in all probability, secondary to the peritoneal stretching during laparoscopy with stimuli for the stress response arising from visceral and peritoneal afferent nerve fibres in addition to those from the abdominal wall.

2. Catecholamine Response:
In response to surgical stress, the levels of Catecholamines (epinephrine and norepinephrine) altered markedly in all the three groups. Pre-op value serum Catecholamines (epinephrine and norepinephrine) was comparable among the three groups (p>0.05).

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 open groups (p<0.001) while epinephrine level returned towards pre-op levels in the laparoscopic group (p>0.05). 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.

Thus, we find in the LC group of the present study that 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). 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 4th 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).

**C. 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 at 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 4th (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 4th day of surgery. However, significant difference between laparoscopic cholecystectomy and open hysterectomy was noted at 1st postop day (p<0.001).

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

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

On the 4\textsuperscript{th} 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 1\textsuperscript{st} post-op day which increased further on the 4\textsuperscript{th} 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-\(\alpha\) 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.

E. Haemodynamic Responses:

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 and to the biliary tract through celiac plexus. Higher branches of the greater splanchnic nerves have been 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.

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 4th day (p<0.05).

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 ETCO2 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. RPP increased during intra-op period in all the three groups and decreased markedly to lower than the pre-op value. In LC group (combined, normotensive 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.

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.

F. 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.

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, increased sympathetic tone resulting from reflex activity, baro-reflexes or vasopressin release secondary to peritoneal stretching/stimulation and decreased venous return to the heart.

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, direct stimulation of sympathetic nervous system or increased sympathetic tone resulting from reflex activity.

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.

G. 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.
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.

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.

These observations support the opinion 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 perioperative immune preservation (Minimal TNF-alpha alterations in intra- and postoperative 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.