5. CHAPTER - II

Comparison of the haematological and biochemical markers between normal control and sepsis patients

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5. CHAPTER – II

Comparison of the haematological and biochemical markers between normal control and sepsis patients

5.1 GENERAL INTRODUCTION:
Free radicals, reactive oxygen species (ROS) are formed during a variety of biochemical reactions and cellular functions, and act as pro-oxidants. The formation of free radicals is normally balanced by antioxidants. Oxidative stress (OS) results from an imbalance between formation and neutralization of free radicals. Various pathologic processes disrupt this balance by increasing the formation of free radicals or decreasing the level of available antioxidants or both. Oxidative stress is a major contributing factor to the high mortality rates associated with several inflammatory and other diseases such as severe sepsis (Andrades et al., 2009). The therapy administered in the initial “golden hours” in severe sepsis is likely to influence the outcome (Dellinger et al., 2008). Effective antimicrobial therapies can improve the outcome of septic shock (Kumar et al., 2006) inflammatory and other diseases such as severe sepsis (Andrades et al., 2011). However, to start therapeutic efforts, an early reliable diagnosis is necessary and such therapy may be expensive or invasive (for example broad spectrum antibiotic treatment, surgical focus removal, intensive care treatment, supportive therapy like recombinant human activated protein C). In the most specialized ICU, mortality rate associated with sepsis is approximately 30%, reaching 50% in complicated infectious processes (Angus et al., 2001). Currently the management of sepsis do not have a diagnostic method to quickly identify the causative agent as well as susceptibility to antibiotics (Becker et al., 2004; Sierra, 2007).

Over the last decade or so various researches have been under taken to identify the most definite and significant marker of sepsis so that early aggressive intervention can be taken to limit the progress of the disease. Although various markers have been indentified, controversy exists regarding their significance (Tang et al., 2007). Probably, instead of a single sepsis marker, multiple markers both haematological and biochemical collectively would be more predictive and sensitive.

Keeping this in view we have studied a number of pathological and biochemical markers and an effort have been made to identify the most significant of them.
This chapter deals with analysis of the various sepsis markers. It is divided into three sections. **Section A** deals with the comparison of haematological parameters between normal control and sepsis patients. **Section B** deals with the comparison of serum biochemical parameters (proposed biochemical markers and serum CRP level) between normal control and sepsis patients. **Section C** deals with the analysis of the change in the serum levels of proposed biochemical markers between normal control and sepsis patients with an effort to identify the most significant biochemical marker.

### 5.2 STUDY DESIGN:

60 adult patients of both sexes with abdominal sepsis (septic peritonitis) were enrolled for the study. Blood samples of these patients were obtained on admission and analysed for evaluation of haematological parameters (Hb gm%, WBC count, and platelet count) and serum biochemical parameters (proposed biochemical markers and serum CRP level). The values were then compared with that of normal control patients \( (n = 15) \) to identify the proposed markers with significant difference by applying students 't' test (unpaired t-test). The markers were then computed to evaluate the maximum percentage change between the normal control and the sepsis patients. The data has been mentioned as mean ± SD and percentage (%). \( p < 0.05 \) has been considered statistically significant.

### 5.3 RESULTS:

**Section A**

The comparison of haematological markers between normal control and sepsis patients:

The WBC count (mean ± SD) in sepsis patients on admission was significantly increased \( (p < 0.001) \) as compared to normal control patients whereas the platelet count significantly decreased \( (p < 0.001) \) in sepsis patients when compared with normal control \( (3.40 ± 0.59 \text{ Lacs/cumm} \text{ versus} 2.36 ± 0.81 \text{Lacs/cumm}) \).

Although the Hb gm% decreased in sepsis patients in comparison to normal control, it did not achieve statistical significance \( (11.10 ±1.66 \text{ gm%} \text{ versus} 10.35 ± 1.87 \text{ gm%}, \text{ p > 0.05}) \). The results are summarized in table - 1 and graphs 1 - 3.
Table – 1

Comparison of different haematological markers between normal control and sepsis patients

<table>
<thead>
<tr>
<th>Parameters (Markers)</th>
<th>Normal control n = 15</th>
<th>Sepsis patients n = 60</th>
<th>Sig (2 tailed)</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb gm%</td>
<td>11.10 ± 1.66</td>
<td>10.35 ± 1.87</td>
<td>0.13</td>
<td>Lower -0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper 1.77</td>
</tr>
<tr>
<td>WBC count (cells/cumm)</td>
<td>6760.00±1555.54</td>
<td>17134.23 ± 3530.88</td>
<td>&lt; 0.001*</td>
<td>Lower -11593.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper -9155.44</td>
</tr>
<tr>
<td>Platelet count (Lacs/cumm)</td>
<td>3.40 ± 0.59</td>
<td>2.36 ± 0.81</td>
<td>&lt; 0.001*</td>
<td>Lower 0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper 1.41</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. 95% Confidence Interval (CI) and the significance was analysed between normal control and sepsis patients, applying unpaired t-test. * = statistically significant at p < 0.001 for WBC counts and platelet counts.

Graph – 1

The level of haemoglobin in normal control and sepsis patients

Results are expressed as mean ± SD of Hb gm% in normal control and sepsis patients.
Results are expressed as mean ± SD. The significance was analysed between normal control and sepsis patients by unpaired ‘t’ test. * = statistically significant at p < 0.001 for increased WBC counts and decreased platelet counts.
Section B
The comparison of biochemical markers between normal control and sepsis patients:

As depicted in table 2 and graphs 4 – 9, the serum level (mean ± SD) of all the proposed serum biochemical markers CRP of sepsis patients on admission was significantly high (p < 0.001) as compared to the normal patients. The total SH group level in sepsis although raised as compared to normal control, did not achieve statistical significance.

Table - 2
Comparison of biochemical markers between normal control and sepsis patients

<table>
<thead>
<tr>
<th>Parameters (Markers)</th>
<th>Normal control n = 15</th>
<th>Sepsis patients n = 60</th>
<th>Sig (2tailed)</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>total SH group (mmoles/L)</td>
<td>2.52 ± 0.46</td>
<td>2.70 ± 0.41</td>
<td>0.18</td>
<td>Lower -0.45</td>
</tr>
<tr>
<td>LPO (μmole/L)</td>
<td>1.71 ± 0.35</td>
<td>13.75 ± 4.87</td>
<td>&lt;0.001*</td>
<td>Lower -13.31</td>
</tr>
<tr>
<td>SOD (Units/mL)</td>
<td>1.81 ± 0.48</td>
<td>7.19 ± 0.95</td>
<td>&lt;0.001*</td>
<td>Lower -5.83</td>
</tr>
<tr>
<td>Catalase (Units/mL)</td>
<td>2.00 ± 0.70</td>
<td>10.82 ± 2.66</td>
<td>&lt;0.001*</td>
<td>Lower -9.59</td>
</tr>
<tr>
<td>GPX (Units/mL)</td>
<td>0.89 ± 0.04</td>
<td>2.21 ± 0.51</td>
<td>&lt;0.05*</td>
<td>Lower -1.45</td>
</tr>
<tr>
<td>GR (Units/L)</td>
<td>8.29 ± 1.27</td>
<td>46.75 ± 5.40</td>
<td>&lt;0.001*</td>
<td>Lower -40.00</td>
</tr>
<tr>
<td>GST (Units/L)</td>
<td>0.67 ± 0.23</td>
<td>4.58 ± 0.63</td>
<td>&lt;0.001*</td>
<td>Lower -4.11</td>
</tr>
<tr>
<td>G6PDH (Units/L)</td>
<td>5.81 ± 1.07</td>
<td>18.01 ± 1.52</td>
<td>&lt;0.001*</td>
<td>Lower -12.89</td>
</tr>
<tr>
<td>LDH (Units/L)</td>
<td>80.44 ± 8.24</td>
<td>318.55 ± 5.80</td>
<td>&lt;0.001*</td>
<td>Lower -274.18</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>7.86 ± 2.29</td>
<td>226.31 ± 50.46</td>
<td>&lt;0.001*</td>
<td>Lower -231.53</td>
</tr>
</tbody>
</table>

Results show the mean ± SD of serum level of different biochemical marker in normal control and sepsis patients. 95% CI and the significance was analysed between normal control and sepsis patients, using unpaired t-test. *p = statistically significant at 0.05 and 0.001
The serum total SH groups in normal control and sepsis patients

![Graph 4](image)

The level of serum LPO in normal control and sepsis patients

![Graph 5](image)

Results are expressed as mean ± SD. The significance was analysed between normal control and sepsis patients by unpaired 't' test. * = statistically significant at p < 0.001 for increased LPO (enzyme activity)
Graph - 6

The level of serum SOD, catalase and GPX in normal control and sepsis patients

Results are expressed as mean ± SD. The significance was analysed between normal control and sepsis patients by unpaired ‘t’ test. * = statistically significant at p < 0.001 for increased SOD and catalase (enzyme activity) and at p < 0.05 for increased GPX (enzyme activity).
The level of serum GR and G6PDH in normal control and sepsis patients

Graph - 7

Results are expressed as mean ± SD. The significance was analysed between normal control and sepsis patients by unpaired ‘t’ test. * = statistically significant at $p < 0.001$ for increased GR and G6PDH (enzyme activity).
Results are expressed as mean ± SD. The significance was analysed between normal control and sepsis patients by unpaired 't' test. * = statistically significant at p < 0.001 for LDH (enzyme activity).
The level of C-reactive protein in normal control and sepsis patients

Results are expressed as mean ± SD. The significance was analysed between normal control and sepsis patients by unpaired ‘t’ test. * = statistically significant at p < 0.001 for CRP level.
Section C

Evaluation of the change in the haematological and biochemical markers between normal control and sepsis patients:

The highest percentage increase among the sepsis markers (haematological and biochemical) was in serum CRP level (2779.26%). Among the proposed biomarkers maximum increase was in serum LPO level (704.10%), followed by GST level (583.58%), GRD (463.93%) and catalase (441%). The rest of the biomarkers increased ranging from 150% to 300% approximately, with a minimum rise exhibited by total SH (7.14%). However, the WBC count increased by 60.5% and Hb gm% and platelet count decreased by 6.76% and 30.59% respectively. The results are summarized in table 3 and graph 10 – 11.

Table – 3

<table>
<thead>
<tr>
<th>Parameters (Markers)</th>
<th>Normal control</th>
<th>Sepsis patients</th>
<th>Difference of mean (Sepsis – Control) (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb gm/dl</td>
<td>11.10 ± 1.66</td>
<td>10.35 ± 1.87</td>
<td>0.75 (-6.76%) †</td>
</tr>
<tr>
<td>WBC count (cells/cumm)</td>
<td>6760 ±1555.54</td>
<td>17134.23 ± 3530.88</td>
<td>10374.23(153.47%) †</td>
</tr>
<tr>
<td>Platelet count (Lacs/cumm)</td>
<td>3.40 ± 0.59</td>
<td>2.36 ± 0.81</td>
<td>-1.04 (-30.59%) †</td>
</tr>
<tr>
<td>CRP level (mg/L)</td>
<td>7.86 ± 2.29</td>
<td>226.31 ± 50.46</td>
<td>218.45 (2779.26%) †</td>
</tr>
<tr>
<td>Total SH group (mmoles/L)</td>
<td>2.52 ± 0.46</td>
<td>2.70 ± 0.41</td>
<td>0.18 (7.14%) †</td>
</tr>
<tr>
<td>LPO (μmoles/mL)</td>
<td>1.71 ± 0.35</td>
<td>13.75 ± 4.87</td>
<td>12.04 (704.10%) †</td>
</tr>
<tr>
<td>SOD (Units/L)</td>
<td>1.81 ± 0.48</td>
<td>7.19 ± 0.95</td>
<td>5.38 (297.24%) †</td>
</tr>
<tr>
<td>Catalase (Units/L)</td>
<td>2.00 ± 0.70</td>
<td>10.82 ± 2.66</td>
<td>8.82 (441%) †</td>
</tr>
<tr>
<td>GPX (Units/mL)</td>
<td>0.89 ± 0.04</td>
<td>2.21 ± 0.51</td>
<td>1.32 (148.31%) †</td>
</tr>
<tr>
<td>GR (Units/L)</td>
<td>8.29 ± 1.27</td>
<td>46.75 ± 5.40</td>
<td>38.46 (463.93%) †</td>
</tr>
<tr>
<td>GST (Units/L)</td>
<td>0.67 ± 0.23</td>
<td>4.58 ± 0.63</td>
<td>3.91 (583.58%) †</td>
</tr>
<tr>
<td>G6PDH (Units/L)</td>
<td>5.81 ± 1.07</td>
<td>18.01 ± 1.52</td>
<td>12.2 (209.98%) †</td>
</tr>
<tr>
<td>LDH (Units/L)</td>
<td>80.44 ± 8.24</td>
<td>318.55 ± 85.80</td>
<td>238.06 (295.95%) †</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. The difference in mean values for each parameter between normal control and sepsis patients was computed. † Values in the parentheses represent percentage change from control.
Graph - 11

The percentage change in serum haematological markers in sepsis patients from normal control

Results are expressed as percentage change. The difference in mean values for each haematological parameter between normal control and sepsis patients was computed and shown as percentage change from control.
The percentage change in the serum biochemical markers in sepsis patients from normal control

Results are expressed as percentage change. The difference in mean values for each biochemical parameter between normal control and sepsis patients was computed and shown as percentage change from control.
Graph - 13

The percentage change in serum level of proposed biochemical markers in sepsis patients from normal control

Results are expressed as percentage change. The difference in mean values for each proposed biochemical parameter between normal control and sepsis patients was computed and shown as percentage change from control.
DISCUSSION:

Haematological Markers:

In the present study the haematological markers, i.e. WBC count significantly increased in sepsis patients as compared to normal control whereas the platelet count significantly decreased in sepsis patients as compared to normal control group. The Hb gm% also decreased but it was not statistically significant. These findings were in accordance to the previous studies.

In 2002 the American College of Chest Physician and the Society Of Critical Care Medicine Consensus conference defined the term Systemic Inflammation Response Syndrome (SIRS) as a response to variety of severe clinical insults manifested by two or more of the following manifestations: 1) temperature of > 38 °C or > 36 °C, 2) heart rate of > 90 beats/min, 3) respiratory rate of > 20 breaths/min or PaCO2 < 32 mm Hg, 4) WBC count > 12000/mm³, < 4000 mm³ or > 10% immature (bands) forms. Sepsis is the systemic inflammatory response to infection. In association with infection the manifestation of sepsis is similar to SIRS.

Abnormal WBC count is therefore one of the pre-requisite for diagnosing SIRS and or sepsis. However, since it can be a manifestation of variety of illness, it is not a specific marker of sepsis. High WBC count usually means there is increase in production of these cells to fight a possible infection. The white blood count is an easily available, inexpensive and a reliable test which enables the doctors to arrive at better prognosis regarding the patient's well-being. However, researchers believe that a cause and effect link between the higher white blood cell count and illness has not been established and they continue to evaluate if the elevated count helps trigger a serious disease or if the cell count rises naturally after an illness (Aminzadeh and Parsa., 2011). Similarly, in our series the WBC count was found raised (> 12000/mm³) in nearly all the sepsis patients.

The WBC counts are usually less in elderly patients as compared to young adults. Aminzadeh and Parsa., (2011), found that even in elderly patients the WBC count is dramatically raised during infection. They conducted a study on 130 patients and found a significant correlation between the source of infection and WBC counts in the elderly group (p < 0.05), i.e. WBC count ≥ 14,000 was more common in urinary tract, gastrointestinal and skin infections. They also found a significant correlation between WBC count and age of the patients (p < 0.05) i.e. WBC ≥ 12,000 was more common
in patients aged 45-64 years, and WBC < 4000 was more frequent in young patients aged less than 30 years.

Blommendahl et al., in 2002 analysed serum CRP level and the blood immature to total neutrophil leucocyte ratio, in neonates. All had reasonable (58-77%) sensitivity, reasonable (62-84%) specificity, good (94-97%) negative predictive value and poor (16-24%) positive predictive value for the diagnosis of sepsis. They were of the opinion that instead of single test a combination of various tests would produce improvements in sensitivity or specificity.

Most clinicians have recognized that platelets are involved in the pathogenesis of sepsis, if only by the fact that marked thrombocytopenia is a common feature of sepsis. In the present study the mean platelet count in the sepsis patients was 2.39 Lac/cumm, which was significantly less than the normal patients. However, only 15% (not shown in the table) of our sepsis patients had platelet count < 1.50 Lac/cumm which was quite less as compared to the previous studies.

The incidence of thrombocytopenia (platelet count <150 x 10^9/L) in critically ill medical patients is 35-44% (Vanderschueren et al., 2000). A platelet count of <100 x 10^9/L is seen in 20-25% of patients, whereas 12-15% of patients have a platelet count <50 x10^9/L. Typically, the platelet count in patients with sepsis decreases during the first 4 days in the intensive care unit (Akca et al., 2002). Sepsis is a clear risk factor for thrombocytopenia in critically ill patients and the severity of sepsis correlates with the decrease in platelet count (Mavrommatis et al., 2000). Housinger et al., (1993), in their review article examined the value of a falling platelet count in predicting the development of sepsis. They found that Thirty-one of the 32 nonsurvivors developed a platelet count less than 100 x10^9/L. Only 10 of the survivors had a similar occurrence. Platelet count decline preceded other signs of sepsis in all cases. A platelet count below 100 x10^9/L for more than 4 days was uniformly associated with death. Their results emphasized platelet count as an independent predictor of sepsis and death.

The mechanism by which thrombocytopenia in sepsis occurs, however, is not completely clear. Impaired production of platelets from within the bone marrow may seem contradictory to the high levels of platelet production-stimulating pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, and high concentration of circulating thrombopoietin in patients with sepsis. Platelet consumption may also play an important role in patients with sepsis, due to ongoing
generation of thrombin (which is the most potent activator of platelets in vivo) (Warkentin et al., 2003). Yaguchi et al., (2004) reported on their studies on platelet function in patients with sepsis. They observed reduced aggregability of platelets collected in blood from patients with sepsis. The decrease in platelet aggregation was related to the severity of sepsis. The result can be interpreted in two ways. It may be that there is a decrease platelet function in sepsis, but a more likely explanation is that an increased platelet aggregation activity in patients with sepsis will lead to circulating platelets that are already activated and will not aggregate in an ex vivo setting. It is highly probable that activated platelets play a role in the generation of thrombin and fibrinogen to fibrin conversion. The alteration of platelet function and count can be observed in septic patients affected by microvascular insufficiency (Winning et al., 2009) According to Lee et al., (1993 a), about 58% of patients may develop thrombocytopenia during severe sepsis while other studies have not found a connection between the actual platelet count and function (Lee et al., 1993 b; Boldt et al., 1994)

Anaemia is very common in acutely ill patients, to the extent that about one-third of ICU patients receive a red blood cell (RBC) transfusion at some point during their ICU stay (Vincent and Piagnerelli, 2006). In our study the mean haemoglobin concentration in sepsis patient on admission was 10.35 mg/dl. It was less than the normal control patients (11.10 gm/dl) but the difference was not statistically significant (Table – 1 and 3). Further, approximately 34% (not shown in the table) of the sepsis patient on admission had haemoglobin less than 10 gm/dl. This was within the range of the previous studies. A large observational study including 3534 patients in Western European ICUs (Vincent et al., 2002) indicated that the mean admission haemoglobin concentration was 11.3 gm/dl, with 63% of patients having an admission haemoglobin concentration less than 12 gm/dl and 29% less than 10 gm/dl. In a prospective monocenter observational cohort study, Chohan et al., (2003), observed that 55% of all patients had a haemoglobin concentration less than 9 gm/dl on admission.

The etiology of anaemia in critically ill patients is multifactorial and includes blood losses (trauma, blood sampling, surgical procedures and occult gastrointestinal bleeding), decreased red blood cell (RBC) production by functional iron deficiency, (Piagnerelli et al., 2006), and altered erythropoiesis (apoptosis of erythroid precursors
and lower erythropoietin concentrations for a given haematocrit) (Dai et al., 1995)
Blood sampling is probably the main factor in the development of anaemia in the
critically ill, especially in patients with sepsis. Indeed, 37 to 65 ml of blood is drawn
daily (Nguyen et al., 2003).
Hemodilution, by abundant intravenous infusions, can also reveal anaemia in the
critically ill. Perhaps a poorly understood cause of anaemia in ICU patients, especially
in septic patients, is the increased uptake of altered RBCs by the reticuloendothelial
system. Indeed, alterations in RBC membrane composition and morphology, such as
seen during the senescence process, can trigger RBC uptake by macrophages of the
spleen and/or the liver. This factor is, however, likely to be a relatively minor cause of
anaemia (Connor et al., 1994).
Inflammatory states are characterized by an increased production of ROS as well as
by a decrease in antioxidant defences. Damage occurs when the production of ROS
exceeds the antioxidant defences of the tissues (Davis et al., 1987). ROS, which
include superoxide anion (O$_2^-$), hydroxylradical (OH$^-$) and hydrogen peroxide (H$_2$O$_2$),
produced by the WBCs can also damage haemoglobin and induce hemolysis
(Uyesaka et al., 1992). ROS can also affect the lipid part of the RBC membrane by
induction of lipid peroxidation. Huet et al., (2007), recently showed that RBC
membrane lipid peroxidation was increased in patients on the first day of septic shock,
as reflected by significantly increased levels of thiobarbituric acid-malondialdehyde
concentrations. Moreover, the antioxidant defenses of these RBCs were also reduced,
as reflected by decreased glutathione content and reduced activities of SOD and
catalase.
There is some suggestion – but no proof in sepsis – that altered RBCs are more
rapidly cleared from the circulation (Piagnerelli et al., 2007). Regrettably, there are no
data concerning a possible increased erythrophagocytosis of RBCs from septic
patients.

C-reactive protein:
In the case of CRP, no definite correlation between infection and change of plasma
concentrations have been documented (Matson et al., 1997). On the contrary, some
authors have reported that daily measurement of serum CRP is useful in the detection
of sepsis and that it is more sensitive than currently used markers, such as bleeding
time (BT) and WBC count (Povoa et al., 1998). In our study all the sepsis patients had
serum CRP level more than 100 mg/mL with a mean value of 226 mg/mL which was approximately 32 times or 2779% more than the normal control value (Table – 2 and 3). The rise was nearly similar to the previous studies as discussed below.

C-reactive protein is an acute-phase protein, the blood levels of which increase rapidly in response to a variety of clinical conditions. The mean concentration of CRP in healthy patients is 0.8 mg/L, but after a stimulus, increases this value up to more than 10,000 times (Amezcua-Guerra et al., 2007). They were of the opinion that CRP levels < 10 mg/L represents slight inflammatory processes, levels from 10 to 100 mg/L means moderate inflammatory processes and severe inflammatory processes have > 100 mg/L like sepsis.

CRP is a useful parameter to determine if a patient presents sepsis even though cultures are negative. Matson et al., (1997), depicted that a 25% rise in the plasma concentration of C-reactive protein in the absence of other non-infective causes of a raised C-reactive protein, such as inflammation, tissue injury or surgery, is highly suggestive of infection, but failure of the C-reactive protein to rise does not eliminate a diagnosis of sepsis. Póvoa et al., (1998), found that a plasma CRP of 50 mg/L or more was highly suggestive of sepsis (sensitivity 98.5%, specificity 75%). They concluded that daily measurement of CRP was useful in the detection of sepsis and it was more sensitive than the markers, such as BT and WBC. A Portuguese study determined that values greater than 148 mg/dl of CRP are definitive to consider sepsis, and values below 24 mg/dl are negative for such process (Povoa, 2002). Most of the studies have demonstrated a value ranging from 80 mg/ml to 150 mg/ml and above as a strong indicative of bacterial infection sepsis (Luzzani et al., 2003; Castelli et al., 2004). In an Australian study a cohort value for the CRP values was obtained, mentioning a 7% risk of death with levels greater than 150 mg/dl and up to 21% when levels are greater than 300 mg/dl, with a mean in this study of 90 mg/dl and a mortality of 4.3% for this value (Seller-Pérez et al., 2005). Lopez et al., (2011), demonstrated a level of 128 mg/ml indicative of a process of bacterial infection and sepsis. The relative risk of mortality with CRP was significant with values greater than 128 mg/dl. When these values are compared with current literature it is possible to affirm that patients with CRP levels greater than 128 mg/dl have a greater risk of death (Lopez et al., 2011).
Similarly in the present study, the rise in the serum CRP level was significantly higher than the normal control. The mean CRP level was 226 mg/dl. Further, among the sepsis marker (both haematological and biochemical), it had the highest percentage rise from the normal value (Table – 2 and 3). As per the previous studies, all these parameters indicated that our patient was septic and had a high risk of death. We could not comment on the 28 day outcome of the patients since it was an interventional study restricted to 3 days of antioxidant therapy in the ICU. However, we can say that rise in CRP was the most predominant and significant feature in our septic patients.

Proposed biochemical markers:
Sepsis is a leading cause of mortality in critically ill patients. Delay in diagnosis and initiation of antibiotics has been shown to increase mortality. However, differentiating sepsis from non-infectious triggers of the systemic inflammatory response syndrome (SIRS) is difficult, especially in critically ill patients who may have SIRS for other reasons. It is this conundrum that predominantly drives broad-spectrum antimicrobial use and the associated evolution of antibiotic resistance in critical care environments. It is perhaps unsurprising, therefore, that the search for a highly accurate biomarker of sepsis has become one of the holy grails of medicine.

In the present study the serum levels of the proposed biomarkers, LPO, SOD, catalase, GPX, GR, GST, G6PDH, and LDH in sepsis patients were significantly higher than the normal control patients. Although the total SH level was increased as compared to control it did not achieve statistical significance (Table – 2).

The significant rise of the antioxidants in our sepsis patients was in accordance to the previous studies. The rise in antioxidants in septic patients could have been due to various mechanisms.

Earlier studies emphasized only on two mechanisms. The activated phagocytes and ischemia-reperfusion injury. Activated phagocytes produce more free radicals in response to infection. On the other hand the transitory circulatory insufficiencies to the heart, lungs, brain, and other organs during sepsis can cause reperfusion/reoxygenation injury and produce free radicals (Werns and Lucchesi, 1990).

However Leff and his colleagues, (1992 & 1993), discussed several other possible mechanisms for the increases in plasma activities of antioxidant enzymes in sepsis,
including hemolysis, increased enzyme production, or decreased clearance. Whereas
Warner and his colleagues, (1995), said that the most reasonable explanation for the
rise in antioxidant enzyme was damage to the various body tissues.
Recently several other mechanisms have been postulated, including the mitochondrial
respiratory electron transport chain, xanthine oxidase activation, the respiratory burst
associated with neutrophil activation, and arachidonic acid metabolism. There now
exists a considerable body of evidence for redox imbalance and oxidative stress in
human sepsis, demonstrating increased markers of oxidative damage, direct evidence
of free radical production using electron paramagnetic resonance analysis, xanthine
oxidase activation, increased redox reactive iron, abnormal handling of exogenous
antioxidants, and low concentrations of individual endogenous antioxidants.
Under normal physiological conditions, a homeostatic balance exists between the
formation of reactive oxidizing / oxygen species and their removal by endogenous
antioxidant scavenging compounds. Oxidative stress occurs when this balance is
interrupted by excessive production of reactive oxygen species, including superoxide,
hydrogen peroxide and hydroxyl radicals, and/or by inadequate antioxidative
defences, including superoxide dismutase (SOD), catalase, vitamins C and E, and
reduced glutathione (GSH). Both may occur in sepsis.
Early work by Takeda and colleagues, (1984), found reduced plasma \( \alpha \)-tocopherol
levels accompanied by increased plasma thiobarbituric acid reactive substance
(TBARS) levels in critically ill patients compared with controls, suggesting increased
lipid peroxidation. Leff et al., (1992), established that septic patients had more \( (p <
0.05) \) catalase activity \( (54.9 \pm 10.9 \text{ U/mL}) \) than control subjects \( (7.3 \pm 0.8 \text{ U/mL}) \)
whereas the glutathione peroxidase (GPX) activity was same in both. Further, Serum
\( \text{H}_2\text{O}_2 \) scavenging activity correlated with serum catalase \( (r = 0.77) \) but not GPX \( (r =
0.33) \) activity. However, in the present study, a rise in GPX activity was observed in
sepsis patients though the magnitude was less as compared to the rise in CAT activity
(Table - 3). Most plausible explanation could be that raised GPX and CAT could
have acted in concert against several fold increase LPO.
In the present series the SOD activity increased by approximately 300% in sepsis
patients in comparison to normal control. Warner et al., (1995), have also reported a
significant rise in SOD \( (P <0.003, n = 32) \), erythrocyte (RBC) SOD \( (P <0.007, n =
16) \), plasma CAT \( (P <0.0001, n = 32) \), and RBC CAT \( (P <0.005, n = 16) \) in septic
patients when compared with healthy adult controls (n = 7). They also tried to establish the prognostic value of the antioxidants by correlating the SOD and CAT level with the survivors and non-survivors. Statistically significant differences were found for total plasma SOD (P <0.05) and plasma CAT (P <0.009) when survivors (n = 15) were compared with non-survivors (n = 17). Of the four analytes measured, total plasma SOD appeared to have the greatest prognostic potential.

Similarly, Koltuksuz et al., (2000), documented significantly higher level of SOD and MDA in patients with acute appendicitis as compared to normal controls. The mean WBC count was significantly higher compared to the normal controls. Alonso de Vega and his colleagues, (2002), reported similar findings in patients with SIRS. They had higher APACHE III scores and plasma concentrations of lipid peroxidation products and nitrites/nitrates and lower plasma concentration of reduced sulfhydryl groups and plasma total antioxidant capacity than patients without the syndrome.

Similar observation was documented in animal model. Ritter et al., (2003), demonstrated lipid peroxidation, protein carbonyls, and superoxide dismutase significantly increased in nonsurvivor septic rats and was predictive of mortality. There was a marked increase in superoxide dismutase activity without a proportional increase in catalase activity in nonsurvivors. They concluded that plasma superoxide dismutase was an earlier marker of mortality. Their finding was similar to Warner et al., (1995).

The oxidative stress following sepsis in children is similar to that of an adult. Batra and colleagues, (2000), found an increased production of ROS in neonates with sepsis, which was witnessed by the positive regulation of XO, SOD and GPX activity. The elevation of antioxidant enzymes, however, was not so effective as to protect from cellular damage and thereby result in higher MDA (a marker of lipid peroxidation) production. They inferred that antioxidant therapy could be useful in the management of neonates with sepsis but also suggested a further detailed clinico-biochemical investigation to define effective antioxidant therapy. In a similar study Kapoor et al., (2006), found significantly higher levels of MDA, SOD, GPX, and catalase, in septicemic patients while the levels of UA and Alb were significantly lower as compared to controls (p<0.001). They concluded that neonates with sepsis are handicapped in terms of their defence mechanism against free radicals.
However, contrary to the previous studies on paediatric patients, Cherian et al., (2007), showed an adaptive response of the body to combat stress in children up to 5 years of age. They found no significant change in erythrocytic GSH, SOD and TBARS levels between sepsis and normal controls. However, plasma vitamin C levels were significantly reduced in patients aged one year one month to five years which may be due to active phagocytosis and due to its role as a free radical scavenger. The activity of GST increased profoundly in septic patients as compared to the normal control (Table – 3). The increase was more than that noted for GPX. Relatively uncharged GPX unlike SOD and CAT in peritonitis patients compared to controls have been explained on the basis of selenium levels (Kumar et al., 2007). It has also been reported that in patients with infection there is an early and marked decrease in plasma selenium due to increased metabolic use and the concentration of GPX is dependent on selenium levels (Forceville et al., 1998). However, in our study the decline in selenium levels could not be the only reason as GPX activity also increased almost more than 100%. Increase in nitrosative stress in sepsis is well known (Gutteridge & Mitchell, 1999). Previous studies have reported activation of GST by peroxinitrite (Ji and Bennet, 2003) and its role in limiting the extent of oxidative tissue injury when other antioxidant enzymes such as SOD are compromised under pathophysiological conditions of excessive nitrosative stress (MacMillan-Crow et al., 1998). This also partly explains a several fold high activity of GST in comparison to other antioxidant enzymes studied. Besides neutralization of hydroperoxides, GST is also entrusted with a job of detoxification of xenobiotics derived from pathogenic microorganisms, catalyzing their conjugation with GSH (Sagara et al., 1998). Thus increase in GST activity might also indicate endogenous detoxification of microbial xenobiotics (Halliwell & Gutteridge, 2007).

Glutathione metabolism is altered in sepsis. Koo and colleagues, (2000), observed an increase in plasma α GST levels earlier than plasma lactate and liver transaminase levels in an animal model. They were of the opinion that α GST may be a more sensitive indicator of early liver injury and should be used in monitoring hepatocellular damage during the progression of sepsis. The enzyme GR has an important accessory antioxidant function related to GPX and GST. GR intervention continuously regenerates GSH from GSSG in presence of
NADPH, thereby preventing loss of GSH (Noctor and Foyer, 1998), explaining the increase in GR activity in sepsis patients in the present study.

Also in animal study conducted by Malmezat and colleagues, (2000), the glutathione synthesis rates significantly increased in liver (+465%), spleen (+388%), large intestine (+109%), lung (+100%), muscle (+91%) and heart (+80%) of infected rats compared with pair-fed controls. Glutathione concentrations were also greater in these tissues but were unaffected in small intestine and lower in blood. In keeping with the stimulation of liver glutathione synthesis, the activities of liver γ-glutamyl-cysteine synthetase and glutathione reductase were significantly greater in liver of infected rats than of pair-fed rats. The authors were of the opinion that glutathione synthesis accounted for at least 40% of the enhanced cysteine utilization during infection.

It has been observed that common type A- G6PD deficiency predisposes septic complications and anemia in trauma patients after severe injuries. Spolarics et al., (2001), reported 50% of the deficient and 6.2% of nondeficient patients developed sepsis with positive bacterial blood cultures in patients with severe injury. In deficient patients, the frequency of bronchial (75%) and wound infections (25%) was also increased compared with nondeficient patients (32% and 0%). The durations of systemic inflammatory response syndrome, Sepsis Syndrome, and days on antibiotics were three times longer in deficient than in nondeficient individuals. However, adult respiratory distress syndrome occurred in 37% of both groups. Similarly, Wilmanski et al., (2007) reported increased interleukin-1beta, interleukin-6, and interleukin-10 levels in serum and peritoneal lavage in G6PD-deficient mice after endotoxemia or polymicrobial sepsis (caecal ligation and puncture). We witnessed an increase in the level of G6PDH in sepsis patients (Table - 2 and 3). This could be an indirect evidence of increased oxidative stress due to sepsis. The increase in G6PDH could be a compensatory mechanism to provide reducing equivalents to GR for GSH regeneration. The reduced glutathione, GSH, is responsible for scavenging free radicals in oxidative stress.

The level of antioxidants and free radicals in plasma has been analysed to predict the severity of sepsis and mortality. Lactate dehydrogenase (LDH) increases in sepsis. It is a sensitive marker of cell injury and reflects the degree of tissue injury and reperfusion (Zein et al., 2004). Zein and his colleagues, (2004), found 79% of their septic patient with increased LDH. Further, they demonstrated an association between
LDH and higher APACHE II score, and significant correlation between LDH and lactic acid levels \((r =0.76; \ p<0.01)\), and between LDH and MODS score \((r =0.6; \ p<0.01)\). They concluded that failure to improve LDH levels at 48 hours was a strong predictor of mortality in patients with severe sepsis.

Ozdogan et al., (2006) investigated the levels of total anti-oxidant status (TAS), and malondialdehyde (MDA) and CRP in patients with acute appendicitis. They concluded that a decrease in plasma total anti-oxidant capacity could be a predictor of the progression of inflammation to the perforation in acute appendicitis.

Similarly, Andrades and colleagues, (2011) in a rat model found a positive correlation between thiobarbituric acid reactive species (TBARS) and markers of organ injury in lung and kidney. Oxidative damage was correlated with an increase in SOD/CAT ratio only in the lung. In contrast, oxidative damage was correlated with myeloperoxidase (MPO) activity in the kidney, but not lung, suggesting different sources of oxidative damage depending on the analyzed organ. Further, antioxidant effects seemed to depend not only on the diminution of oxidative damage but also on its anti-inflammatory activity.

From the above discussion it can be inferred that our study was in accordance to the previous studies and sepsis patients had significant rise in haematological and biochemical markers except Hb gm%, platelet count and total SH group as compared to the normal control patients.

However, some of the factors which could have influenced our results were – 1) demographic profile of the sepsis patients (gender, age, weight), 2) severity of sepsis/infection, 3) duration of infection, 4) volume status of the patients, 5) anaemia/Hb gm% of the patients, 6) renal or hepatic failure, 7) technique of sample collection

The gender, age, and weight of both the sepsis and the normal control patients were comparable in our study. Individually they might have influenced the results by either increasing or decreasing the oxidative stress but since sample population were comparable with each other, they did not influence the overall result (discussed in Chapter – I).

The severity of sepsis has been assessed by evaluating APACHE II scoring. Previous studies have demonstrated that severe sepsis/infection causes severe oxidative damage which subsequently leads to increased level of ROS and decreased level of
antioxidants (Pascual et al., 1998; Metnitz et al., 1999; Ozdogan et al., 2006). In addition, certain ROS (LPO, MDA) indicated massive oxidative stress (Metnitz et al., 1999; Ritter et al., 2003; Zein et al., 2004) and SOD as a prognostic marker. In the present study the APACHE II scoring (mean ± SD) in sepsis was 8.08 ±2.73 which predicts a mortality of approximately 8%. Although all the markers are significantly raised in sepsis patients except Hb gm%, platelet count and total SH group, the markers with maximum change from normal was CRP, LPO, LDH and GR (Table – 7, Graph – 8). In addition the 95%CI of these markers have a wide/broad spread indicating a definite rise in most of the patients. This was in accordance to the previous authors (dealt in detail in Chapter III).

Duration of infection could be an indirect evidence of degree of injury/damage. It may be assumed that increased duration of illness would lead to increased prostration and decrease physiological reserve and immunity. Studies have documented that duration of infection can influence the levels of ROS and antioxidants (Llesuy et al., 1994; Pascual et al., 1998; Metnitz et al., 1999; Koo et al., 2000; Ritter et al., 2003). In our study the sample was collected on the admission day from all patients, according to the protocol. However, the duration of infection could have been different in each patient depending upon the delay in admission to the hospital. Enrollment of the patient and sample collection was considered as the beginning of the study (preoperative sample/admission sample) and was kept constant for all patients. Hence we assume that duration of infection was same for all patients and did not influence our overall result.

Intravascular volume status of the patients could influence the plasma level of the antioxidants. Over hydration could have led to false decrease in the level of the antioxidants in plasma due to haemodilution. The sepsis (septic peritonitis) patients in our study have been resuscitated with fluids to maintain volume status. Fluid resuscitation must have affected all the sepsis patients uniformly if at all. However despite fluid resuscitation there was no significant haemodilution since the Hb gm% between the normal control and sepsis patients were comparable. Further, the results of our study were similar to the previous studies conducted on sepsis patients. The previous studies must have had similar scenario as ours. Hence we assume that fluid resuscitation must have not influenced the overall result. However, there is no literature on this subject to substantiate our view.
Sepsis patients suffer from anaemia (decreased Hb gm%) due to coagulopathy, bone marrow depression, haemolysis, toxins, hemodilution etc. One of the mechanisms for increased antioxidant activity in sepsis is through hemolysis of RBC (Leff et al., 1992). Accordingly, this could have been one of the reasons for increased antioxidant activity in our sepsis patients as compared to normal control. However, whether anaemia/decreased Hb gm% itself influences the antioxidant activity could not be assessed since it was not within the purview of this study. Moreover, there is no study in the literature mentioning anemia to influence antioxidant activity. Therefore, we assume that probably anaemia did not influence our overall result because the Hb gm% was comparable between sepsis and normal control patients.

Increased generation or decreased clearance of ROS is one of the mechanisms of increased plasma antioxidant activity. Therefore, hepatic failure or renal failure could affect the clearance of the ROS and indirectly influence the antioxidant activity. However, this did not influence our result since patients with hepatic failure or renal failure were not included in our study.

Defective technique of blood collection can result in false high level of antioxidant. Blood collected through very narrow bore needle can cause haemolysis of RBC. Improper separation of serum from the cells can also influence the result. However, since a standard and uniform technique was applied for all the sample collection both in normal control and sepsis patients, we assume that this did not influence our result.

From the above results and discussion it can be inferred that CRP level is the most significant and predominant biochemical marker of sepsis in our study. Proposed biomarker levels definitely increase due to oxidative stress in sepsis patients. Most of them increase by more than 100% with LPO having the highest increase. The maximum percentage rise in the serum LPO level is probably an indicator of the massive oxidative damage due to the acute septic peritonitis. This acuteness of the disease process in our patient population was further substantiated by the significant rise in the WBC count and the highest rise of CRP level.