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SUMMARY AND CONCLUSION

Sepsis is a challenge in medicine. The diagnosis can be difficult despite all the laid down criteria since the clinical signs (tachycardia, leucocytosis, tachypnoea, and pyrexia) often overlap with other non-infectious causes of systemic inflammation such as trauma, surgery and hypoxia.

Various markers have been proposed over the years. Numerous studies have documented that reduced plasma enzymatic and non-enzymatic antioxidants are an indirect evidence of increased oxidative stress. Lower levels of antioxidants and greater production of free radicals suggested that reactive oxygen species (ROS) generation play a key role in the outcome of sepsis patients. However, unfortunately, no marker is 100% specific for sepsis and diagnosis must, at present, therefore depend on the presence of a combination of clinical, haematological, and biochemical markers.

To combat the threat of oxidative stress, there exist a number of endogenous antioxidant defences. Vitamin C (ascorbic acid) is a powerful electron donor, reacting with both superoxide and hydroxyl radicals. N-acetylcysteine (NAC), a sulphydryl donor can replete intracellular GSH by donating cysteine. NAC alone and in combination with other antioxidants has been shown to have variable results.

Hence, the aim of the present study was to evaluate 1) the most significant of the proposed markers in sepsis, 2) the correlation of the level of markers with that of the severity of sepsis, 3) the most effective antioxidant agents (N-acetylcysteine and vitamin C or combination) most effective in reducing the level of markers in sepsis.

The present study titled “Evaluation of the effects of N-acetylcysteine and vitamin C on the oxidative stress following abdominal sepsis”, was conducted in the Intensive Care Unit of the Department of Anaesthesiology, J.N. Medical College, AMU, Aligarh and the biochemical analyses was performed in the Dept of Biochemistry, F/O Life Sciences, AMU, Aligarh.

After obtaining approval from the Board of studies, 60 patients suffering from septic peritonitis, posted for laparotomy and 15 elective patients (normal control), a total of 75 patients, of either sex, age ranging between 18 - 60 years, were included in the study. Written informed consent was obtained from the patient or their relatives. Patients with history of un-recordable pulse or blood pressure, acute coronary syndrome, active gastrointestinal hemorrhage, seizure, drug overdose, poisoning, burn...
injury and malignancy were not included in the study. The sepsis patients were divided into four groups (groups B, C, D and E) randomly, each comprising of 15 patients depending upon the antioxidant administered:

- **Group B** – post surgical septic peritonitis patients who received with 100 ml of 5% dextrose 8 hourly
- **Group C** – post surgical septic peritonitis patients receiving N-acetylcysteine @ 70 mg/Kg dissolved in 100 ml of 5% dextrose 8 hourly
- **Group D** – post surgical septic peritonitis patients received Vitamin C @ 25 mg/Kg dissolved in 100 ml of 5% dextrose 8 hourly
- **Group E** – post surgical septic peritonitis patients received a combination of N-acetylcysteine @ 70 mg/Kg and Vitamin C @ 25 mg/Kg dissolved in 100 ml of 5% dextrose 8 hourly

- 10 ml of blood was withdrawn with 10 ml disposable syringe from peripheral veins of all those enrolled in the study. Sample was collected only once from the normal control group (group A) to estimate the control values of the variables in the general population. From the sepsis patients (groups B, C, D, and E) blood was collected on the following days:
  - after admission: prior to any surgical intervention (preoperative sample)
  - post operative day 1 in ICU
  - postoperative day 4 in ICU (after completion of total 9 doses).

For the biochemical analysis the sera were separated from blood as soon as possible by centrifugation at 2000 x g at 4°C for 10 min in Beckman J2-M1 (Beckman instruments. Inc Palo Alto, C.A. USA) refrigerated centrifuge. The serum obtained was stored in aliquots at -20°C until further analysis.

The study ended on the fourth day after collection of the third (last) sample that was one day after the administration of the ninth dose. In situations when the patient expired or required ventilator support postoperatively, after collection of the first sample, the patient was excluded from the study and a fresh case was included in the same group.

- All the patients in various study groups were evaluated clinically and all routine investigation was done. All patients received same standard therapy
- The parameters recorded from each patients were as follows:
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1) APACHE II score within 24 hours of admission,
2) The haematological markers - i) Hemoglobin levels, ii) White blood cell (WBC) count, iii) Total Platelet count,
3) C-reactive protein levels (CRP) and
4) The proposed biochemical markers i) total sulfhydryl (SH) groups, ii) lipid peroxidation (LPO) levels, iii) superoxide dismutase (SOD) levels, iv) catalase levels, v) glutathione peroxidase levels (GPX), vi) glutathione reductase (GR) levels, vii) glutathione-S-transferase (GST) levels, viii) glucose 6 phosphate dehydrogenase (G6PDH) levels, ix) lactate dehydrogenase (LDH) levels. The enzyme levels correspond to their activities.

- All enzymes were assayed at zero order kinetics unless otherwise specified.
- The activities of each enzyme from various comparing groups were determined simultaneously under similar conditions by using same solutions to avoid day to day experimental variations.
- One unit of enzyme activity is defined as the amount of enzyme required to catalyze the formation of 1 μmole of product per min or hour under the specified experimental conditions.

Most of the research has some element of biasness. However, we tried to overcome biasness by initially randomizing the study and subsequently making the study blind. Block randomization was done so that equal number of patients would fall in each group. Blinding was assured by making appropriate dilutions such that equal volumes of study drug based on patient weight were administered. Study drug was administered over 30 min, every 8 hourly, for a total of 9 doses during a 3-day treatment period. Neither the researcher who collected the sample nor the patient was aware of the drug administered. The researcher who collected the sample gave a code number to the sample and maintained the record of the samples sent (name, date & code no.).

The observer who was not the part of the study prepared and administered the drug. He also simultaneously maintained a record of the name of the patient, code no. and the group. The samples were sent for the investigations with a separate code number for each sample so that the data analyser was blind to the name of the patient and the day of the collection. The results of all the samples were sent back by the analyser after the completion of the study.
The data was statistically analyzed using the SPSS 17 software. The significant variation in the level of the markers (both haematological and biochemical) in sepsis patients were compared with the normal control subjects using unpaired t-test. The relationship of the markers with the severity of sepsis (APACHE II) was analysed applying Pearson's correlation co-efficient. Subsequently linear regression analysis was done to understand the statistical dependence of one variable on other variables. The effect of the surgery and study drugs (antioxidants) on the level of the sepsis markers within the groups was analysed applying paired 't' test. To identify the group having the most significant effect on the level of the markers, the difference in the effect of drugs within the groups was computed and one way ANOVA test followed by Post hoc Tukey test was applied. The thesis was divided into four chapters.

**Chapter – I** dealt with the demographic profile of the subjects to identify whether the gender, age and weight of the patients influenced the variations in the level of the haematological and the biochemical markers in sepsis patients. This chapter was subdivided into three sections.

**Section A** compared the distribution of gender of the patient population between normal control and sepsis group. The distribution of male and female patients in normal control subjects was 53% and 47% in comparison to 53.3% and 46.7% respectively in sepsis patients. After randomly dividing the sepsis patients into different groups 47% males and 53% females were in group B, 60% males, 40% females in group C, 60% males and 40% females in group D, and in group E 47% males, 53% females. The groups were comparable with each other.

**Section B** compared the distribution of patients in different age range and the mean ± SD of age between normal control and sepsis patients. The percentage of patients in the age range of 30-40 years, 40-50 years and 50-60 years were 26.6%, 46.8% and 26.6% respectively in normal control subjects in comparison to 23.3%, 38.3% and 38.3% respectively in sepsis patients. The age (mean ± SD) of the patients were 44.13±5.66, 47.06±7.59, 47.2±7.68, 46.26±9.11 and 48.66±8.36 in Group A, Group B, Group C, Group D and Group E respectively. The distribution of patients in the respective age range and the mean ± SD of age between the groups were comparable.

**Section C** compared the distribution of patients in different weight range and the mean ± SD weight between the normal control and sepsis patients. The percentage of
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patients in the weight range of 40-50 kg, 50-60 kg and 60-70 kg were 13.3%, 46.6% and 40 and in sepsis patients it was 10%, 43.3% and 46.6% respectively. The mean (± SD) of the patients in different groups were 56.73 ± 6.09, 57.06 ± 8.53, 61.6 ± 7.55, 61.33 ± 7.47 and 62.86 ± 6.64 in Group A, Group B, Group C, Group D and Group E respectively. The distribution of the patients in the respective weight range and the mean ± SD of the weight between the groups were comparable.

Chapter II dealt with the variations in the levels of different haematological (Hb gm%, WBC count and platelet count) and biochemical markers (proposed biomarkers and CRP) in sepsis patients to that of the normal control. The difference in the levels of the markers between the normal control and sepsis patients were then computed to identify the marker with maximum percentage change. This chapter was subdivided into three sections.

Section A compared the haematological markers between normal control and sepsis patients. The WBC count and the CRP level (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. Although the Hb gm% decreased in sepsis patients in comparison to normal control, it did not achieve statistical significance.

Section B compared the serum proposed biomarkers along with CRP level between normal control and sepsis patients. The serum level (mean ± SD) of all the proposed biochemical markers (enzymatic and non-enzymatic antioxidants) and CRP of sepsis patients on admission was significantly high (p < 0.001) as compared to the normal patients. The total SH level in sepsis although raised as compared to normal control, did not achieve statistical significance.

Section C evaluated the change in the haematological and the biochemical markers between normal control and sepsis patients. The highest percentage increase was given by serum CRP level (2779.26%). Among the proposed biomarkers maximum increase was in serum LPO (704.10%) followed by GST (583.58%), GR (463.93%) and catalase (441%) level. The rest of the biomarkers increased ranging from 150% to 300 % approximately except WBC count (60.5% increase), with a minimum rise by
total SH (7.14%). However, Hb gm% and platelet count decreased by 6.76% and 30.59% respectively.

Chapter III dealt with evaluation of the relationship between the severity of sepsis and the level of sepsis markers (both the haematological and biochemical). The severity of sepsis was assessed by using the APACHE II scoring system. The worst score was then correlated with the level of the sepsis markers. Subsequently the association between the dependent variable (sepsis markers) with that of the independent variable (APACHE II) was analysed applying linear regression. The patients were again divided into two groups on the basis of APACHE II score a) patients with APACHE II < 10, b) patients with APACHE II ≥ 10. The correlation and the association was again analysed between the markers of sepsis and APACHE II score. This chapter was subdivided into two sections.

Section A analysed the correlation between severity of sepsis (APACHE II) and sepsis markers. The APACHE II scores (mean ± SD) of all the sepsis patients together was 8.08 ± 2.73. There was a significant positive correlation (r) between the mean APACHE II score of all the patients and the sepsis markers, except Hb% and Platelet count. The magnitude of relation ranged from weak (r = 0.3) to strong (r = 0.63). The relationship between APACHE II and WBC was strong (r = 0.63) whereas the relationship between LPO, GR and LDH was moderate (r = 0.4 - 0.59).

The association between mean APACHE II score and the sepsis markers was significant and linear, except Hb% and platelet counts. The proportion of variation in the sepsis markers was due to the proportion of variation in APACHE II scores. The variation of the sepsis markers ranged from as low as 5% to as high as 39%.

Section B evaluated the correlation between severity of sepsis of different grades (APACHE II score < 10 and ≥ 10) and sepsis markers. There were 43 patients with APACHE II score < 10 (6.95 ± 1.88) and 17 patients with APACHE II score ≥ 10 (11.23 ± 1.95). Patients with mean APACHE II score < 10 had significant positive correlation (r) with their Hb gm% and LPO levels. The association was also significant and linear. Whereas there was no association between patients with mean APACHE II score ≥ 10 and their haematological and biochemical markers.
Chapter IV dealt with the randomization of the 60 sepsis patients into four groups of 15 patients each. The groups were formed on the basis of drugs (antioxidants) administered. Group B was placebo control, group C received NAC, group D received vitamin C and group E received NAC plus vitamin C. The dose of the drugs was as per the protocol mentioned above. The influence of surgery and antioxidants on the level of sepsis markers was evaluated by comparing between preoperative and postoperative day 1 levels and between postoperative day 1 and day 4 respectively. To identify the group causing most significant change in the levels of sepsis markers, the difference in the level of sepsis markers between day 4 and day 1 was computed and compared between the groups applying one-way ANOVA followed by Post hoc Tukey test. This chapter was subdivided into three sections.

Section A compared the haematological and biochemical markers between the preoperative level and the first postoperative day (day 1) within the groups B, C D and E. Among the haematological markers the Hb gm% and the platelet count did not show significant change on day 1 in comparison to their preoperative levels in all the groups. The WBC count increased on the first postoperative day but was not significantly different than the preoperative value. The level of the biochemical markers (serum CRP, total SH, LPO, SOD, catalase, GPX, GR, GST, G6PDH and LDH) increased on day 1 in comparison to their preoperative level in all the groups. However they did not achieve statistical significance except serum CRP and SOD level, which showed significant increase.

Section B compared the haematological and biochemical markers between postoperative day 1 and day 4 within the groups B, C D and E. There was a significant decrease in WBC count in all the groups (p < 0.01). Although the platelet count and Hb gm% increased in all the groups they did not achieve statistical significance. All the biochemical markers (serum CRP, total SH, LPO, SOD, catalase, GPX, GR, GST, G6PDH and LDH) significantly decreased (p < 0.01) in groups C, D and E. While in group B (placebo control) the level of the markers did not change significantly.

Section C compared the haematological and biochemical markers between the groups to identify the group with most significant change. The difference in the level of sepsis markers between day 1 and day 4 within each group was computed and then compared between the groups. The difference in WBC count, serum CRP, total SH,
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LPO, SOD, catalase, GPX, GR, GST, G6PDH and LDH between day 4 and day 1 was significantly more in groups C, D and E as compared to group B (p < 0.05 to 0.001). While the change in Hb gm% and platelet count between day 4 and day 1 was not significant between groups B, C, D and E.

The change in the level of the haematological and biochemical markers was not significant when compared between group C, D and E although percentage change in the levels was more in group E.

Although the serum level of haematological and biochemical markers decreased in groups C, D and E, they did not reach the control value. The percentage difference to reach the normal value was more in groups B, C, and D as compared to E.

We therefore conclude that the enzymatic and non-enzymatic antioxidant (proposed biomarker) levels definitely increase due to oxidative stress in sepsis patients. Most of the proposed biomarker increase by more than 100% with LPO exhibiting the highest increase. The maximum percentage rise in the serum LPO level is probably an indicator of the massive oxidative damage in 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 CRP level.

The gender, age and weight of the patients can influence the level of haematological and biochemical markers in patients with sepsis. However, since the demographic profile of the patients in our study was comparable with each other, it did not influence the overall result.

All the proposed biomarkers along with CRP level and WBC count have a significant positive correlation and linear association with patients in early sepsis. LPO is the most predominant and significant biomarker that can be useful in assessing the severity of sepsis. However, their role in higher APACHE II score or late sepsis probably needs further study with a larger sample size.

The antioxidants N-acetylcysteine and vitamin C individually can significantly reduce the oxidative stress following sepsis. Their effect is similar to each other. When a combination of N-acetylcysteine and vitamin C is administered it can further reduce the oxidative stress although not significantly different from the individual antioxidants. However, three days of antioxidant administration is not adequate course of therapy to alleviate the oxidative stress and bring it back to normal in post surgical
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septic peritonitis patients. The surgical stress in addition to sepsis probably requires prolonged therapy.

Hence the clinical implication of our observations would be:

1. Serum LPO level determination may be added to the existing battery of investigations to assess the oxidative damage and the severity of sepsis.
2. Antioxidants can be implemented as an adjuvant therapy in the management of abdominal sepsis (septic peritonitis).
3. Administration of vitamin C is as good as N-acetylcysteine. Since the cost of vitamin C is cheaper than N-acetylcysteine, it can be a poor man’s antioxidant.
4. Combination therapy (N-acetylcysteine and vitamin C) may be administered to expedite recovery from oxidative stress in sepsis.
5. Patients undergoing laparotomy following septic peritonitis might need prolonged therapy to combat the oxidative stress.