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Study of Inflammatory Markers and Oxidative Stress in Critically ill Patients with Sepsis

Badade ZG[1], Kavita More[2], Jadhav TS[3], Vyas NL[4]

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Abstract

Introduction

Sepsis is the systemic inflammatory response to infection and remains primary cause of death from infection. Inflammatory markers and oxidative stress has a main role in the pathophysiology of sepsis.

Aims and objectives

The present study aimed to assess oxidative stress markers (MDA, NO, Vitamin C, SOD, CAT), pro inflammatory markers (IL-6 & IL-8 and CRP) and anti-inflammatory marker (IL-10) in septic patients.

Methodology

The study included 100 subjects which were divided into two groups. Group 1 (n=60) included ICU patients diagnosed with sepsis. Group 2 (n= 40) Healthy volunteers. Blood Samples were collected from healthy individuals and from septic patients. The oxidative stress markers (MDA, NO, SOD, CAT and Vitamin C levels were measured by spectrophotometer. IL-6, IL-8 were estimated by ELISA and CRP by Quantitative Turbidimetric Latex Assay Method

Results

The mean serum levels of pro inflammatory cytokines (IL-6, IL-8, CRP) and anti-inflammatory IL-10 septic patients were highly significantly increased in septic patients as compared to control subjects. We found mean serum oxidative stress markers (MDA, NO, SOD and CAT) levels were significantly increased in septic patients as compared to control subjects. Mean serum Vitamin C levels were significantly decreased in septic patients as compared to control subjects ($p<0.05$)

Conclusion

Our study suggests infection and inflammation in septic patients leads to the production of pro inflammatory cytokines, ROS and RNS. This lead to imbalance in the inflammatory network and initiates oxidative stress induced lipid peroxidation of membrane lipids, which is mainly responsible for cellular or organs injury that's confirmed by elevated levels of SOD and CAT. Therefore these pro and antiinflammatory and oxidative stress markers are definitely used as a marker for SIRS and to assess progression and severity of the sepsis.

Keywords: Sepsis; Oxidative stress; Inflammatory markers.

Introduction

Sepsis, or the invasion of microbial pathogens into the bloodstream, is characterized by a systemic pro inflammatory response, which can lead to severe sepsis and septic shock[1]. Sepsis, severe sepsis, and septic shock are foremost healthcare problems globally; they have an effect on millions of people each year, and their prevalence increases annually.[2,3] In spite of significant advances in intensive care units since several years, septic shock remains linked with high mortality rates.[4]

Pathogenesis of sepsis, septic shock and multiple organ failure involves several molecular mechanisms of inflammation and cellular damage, including those related to the generation of

Author's Affiliation: [1]Professor, [2]Associate Professor, [3]Postgraduate student, Department of Biochemistry, [4]Associate Professor, Department of General Surgery, MGM Medical College, Kamothe, Navi Mumbai-410209, India.

Corresponding Author: Badade ZG, Professor, Department of Biochemistry, MGM Medical College, Kamothe, Navi-Mumbai-410209, India.

E-mail: badadezg@gmail.com

cytokines, eicosanoids and reactive oxygen species (ROS) and reactive nitrogen species (RNS).[5,6,7] The anti-inflammatory mechanisms consist, the control of immune response and to maintain system homeostasis.[8] A complex cascade of interactions occurs between different pro and anti inflammatory cytokines and possibly other factors of the immune system, during sepsis; Therefore evaluation of these markers gives an in sight to the clinicians regarding the immune statusof the septic patients to implement correct treatment strategies to overcome the severity of sepsis.

Thus in view of significant role of pro, anti-inflammatory markers and oxidative stress in the in the course of sepsis and very scarcely studied together in septic patients, therefore we aimed to assess oxidative stress markers (MDA, NO, Vitamin C, SOD, CAT), pro inflammatory markers (IL-6 & IL-8 and CRP) and anti-inflammatory marker (IL-10) in septic patients.

Material & Methods

The present study is the prospective study which was carried out in the Department of biochemistry, MGM Medical College and MGM Group of Hospitals Navi Mumbai. We enrolled male and female patients ages of 18 to 80 yrs diagnosed with sepsis, admitted in the ICU of MGM Medical College, Kamothe and MGM Group of Hospitals at Vashi and CBD. Institutional Ethical clearance was obtained and written informed consent was taken from either from patient or patient's closest relative. Patients were recruited between June 2012 and June 2014.

The study design included 100 subjects which were divided into two groups. Group 1 (n=60) included ICU patients diagnosed with sepsis. Group 2 (n= 40) Healthy volunteers.

Sepsis patients were selected from ICU. The diagnosis was based on the presence the following criteria: Sepsis was defined as suspected or confirmed infection in addition to SIRS (that is, presence of pyrexia, tachycardia, tachypnea and/or leukocytosis) Severe sepsis was defined as sepsis with organ dysfunction (hypotension, hypoxemia, oliguria, metabolic acidosis, and/or thrombocytopenia). Septic shock was defined as severe sepsis with hypotension despite adequate fluid resuscitation.

Sample collection

Blood samples were collected from septic patients and healthy individuals. Blood was centrifuged at 3,000 rpm for 10 minutes to obtain serum which was stored at -70° C till analyzes

Oxidative Stress Markers and Pro Inflammatory Cytokines Analyzes:

The following parameters were measured in serum spectrophotometrically using standard methods: Malondialdehyde (MDA) by Satoh's K method[9], nitric oxide (NO•) by Najawa KC and Nabil WW method[10], superoxide dismutase (SOD) by Marklund and Marklund method[11], catalase (CAT) by K Sinha method[12] and vitamin C by DNPH method[13]. IL-6, IL-8, IL-10 were estimated by ELISA (AviBion by Orgenium Laboratories, catalog number IL06001 and IL08001&IL10001respectively) and C-reactive protein by Quantitative Turbidimetric Latex Assay Method[14]

Results

The mean serum levels of pro inflammatory cytokines (IL-6, IL-8, CRP) and anti-inflammatory IL-10 septic patient's were highly significantly increased in septic patients as compared to control subjects ($p<0.0001$).

We found mean serum oxidative stress markers (MDA, NO, SOD and CAT) levels were significantly increased in septic patients as compared to control subjects.

Mean serum Vitamin C levels were significantly decreased in septic patients as compared to control subjects ($p<0.05$)

Table 1: Shows comparison of pro-inflammatory cytokines (IL-6, IL-8, and CRP) and anti-inflammatory IL-10 control group and septic patients

Parameters	Control Group(n=40) mean± SD	Septic patients(n= 60) mean± SD
IL-6 pg/ml	8.23 ± 8.32	260.97 ± 257.68**
IL-8 pg/ml	10.14 ± 7.67	284.105 ± 119.36**
IL-10pg/ml	5.29 ± 6.35	70.28 ± 77.29**
CRP(mg/l)	0.69 ± 0.22	7.13 ± 1.47**

Highly Significant **($p<0001$), Significant *($p<0.05$)

Table 2: Shows comparison of oxidative stress markers (MDA, NO, SOD, CAT and Vitamin C in control group and septic patients

Parameters	Control Group (n=40) mean± SD	Septic Patients (n= 60) mean± SD
MDA (nmol/L)	2.19 ± 0.43	7.85 ± 0.82 **
NO• (µmol/L)	30 ± 7.42	72.52 ± 7.34 **
SOD (U/L)	1.41 ± 0.26	4.89 ± 0.83*
CAT (U/mg of protein/ml)	3.59 ±0.64	8.58 ± 0.92*
Vitamin C (mg/dl)	1.78 ± 0.24	0.43 ± 0.19*

Highly Significant **(p<0001), Significant *(p<0.05)

Discussion

In the present study, we investigated the proinflammatory, anti-inflammatory markers and oxidative stress markers in septic patients and healthy individuals.

Pro Inflammatory and Anti-Inflammatory Marker:

We found the levels of pro inflammatory markers IL-6, IL-8, CRP and anti-inflammatory marker IL-10 were significantly increased ((p<0.0001) as compared to control. Our results are supported by. Gogos CA et al[15],Harbarth S et al[8], Sikora JP et al.[16]

Gogos CA reported that the serum levels of pro inflammatory cytokines (IL-6, IL-8) increases in the course of sepsis in newborns and infants. In response to these increases, anti-inflammatory cytokines (IL-10, IL-13) are increased. Sikora JP et. al.[16] demonstrated both the inflammatory and anti-inflammatory response highly elevated in patients with severe sepsis, the sustained overproduction of the anti-inflammatory cytokine. IL-10 is the main predictor of severity and fatal outcome. HiraoY[17] reported that IL-8 concentration in patients with septic syndrome was significantly (P<.05) higher than in control subjects and suggested that IL-8 is a useful early indicator of bacterial infection.

In the present study Pro-inflammatory cytokines IL-6 IL-8 and CRP are increased due to infection for initiating an effective inflammatory process whereas anti-inflammatory cytokines are elevated in response to control and down regulate the inflammatory response and positive correlation among them proves it. Therefore these pro and anti-inflammatory markers are certainly used as a marker for SIRS and to assess progression and severity of the sepsis.

MDA

We found mean serum MDA levels were significantly increased in septic patients as compared to control subjects (p<0.0001). Similar observations were made by Lorente L et al, [18]Takeda K et. al.[19] Ogilvie A C et al,[20] and Diana Muhl et. al. [21]

Several mediators of sepsis progression, such as LPS, IL-1, IL-6 and TNF-alpha are able to induce NADPH oxidase assembling, xanthine oxidase activity and mitochondria impairment, with consequent superoxide release from cells of the immune system and vascular system. In addition, the release of some cytokines is stimulated by ROS signaling. Sepsis and endotoxemia have been described to induce an imbalance between free radicals generation and its consumption by antioxidant defenses, thus creating a state of oxidative/nitrosative stress to the host at the onset of sepsis. In fact, various researchers have shown that ROS production and oxidative damage occurs early in sepsis and may be used to predictmortality[22]

Takeda K et. al.[19] found the mean plasma α-tocopherol level was significantly lower and the mean TBARS level was significantly higher in critically ill patients as compared to control. 8 ICU patients developed DIC; the mean TBARS level during DIC was significantly above the mean pre-DIC level. These results indicate that LP may contribute to the development of DIC in critically ill patients.

Ogilvie AC et. al.[20] reported higher MDA serum levels in septic patients as compared to controls. They reported MDA levels were higher in non-survivors than in survivors (p<0.05). Further they concluded that an oxidant/antioxidant imbalance, as indicated by elevated plasma lipid peroxides and depressed antioxidants, is involved in human septic shock and a fatal outcome

Our study suggests that the lipid peroxidation in septic patients is highly increased as a result of ROS production due to inflammation and infection, which is responsible for cellular or organs injury or septic shock associated with septicemia.

NO.

We found mean serum NO• levels were significantly increased in septic patients (pre infusion of SMOF) as compared to control subjects (p<0.0001). Our study is supported by Ochoaet al[23] Moncada et al[24], Titheradge et al[25] and Jennyfer B et. al.[26]

Moncada et. al.[24] have linked the production of nitric oxide (NO•) to endotoxin-induced hypotension, vascular hyporesponsiveness and

death, suggesting that the excess production of NO• plays an important role in the development of septic shock. The NO• radical functions efficiently as a mediator, messenger or regulator of cell function in various physiological systems and pathophysiological states. Titheradge et. al.[25], reported that, NO in the pathophysiology of septic shock and MODS and the induction of NO synthase, with the consequent excessive formation of NO, has been proposed to be a major factor involved in the pathologic vasodilatation and tissue damage.

Jennyfer B et. al.[26] demonstrated increased production of NO• in both experimental and clinical infection, inflammation, and sepsis. Although NO• is clearly involved in the development of hypotension during septic shock, further they state that macrophages, or immune cells in general, are the principle source of high systemic NO• levels during septic shock.

The endothelium–derived NO• (EDNO) plays a role in the regulation of blood pressure and tissue perfusion by maintaining vasodilatory tone. Upon stimulation by endotoxins (LPS) and cytokines such as TNF and IL-l, an inducible form of NO synthase (iNOS) is formed in macrophages and vascular smooth muscle cells. This inducible enzyme differs from the constitutive isoform in that it releases massive amounts of NO• resulting in profound vasodilatation with hypotension, dysfunction of vascular autoregulation, resistance to catecholamines and tissue damage.[24,27]

In septic patients upon stimulation by endotoxins and cytokines, an iNOS may formed in macrophages and vascular smooth muscle cells releases massive amount of NO• resulting in tissue damage.

Superoxide Dismutase (SOD) and CAT

We found that mean serum SOD and CAT activity was significantly increased in septic

patients as compared to control subjects Our study is supported by Ann Warner et. al.[28], Seema et. al.[29], Batra et. al.[30] and Kapoor K et. al.[31]

Ann Warner et. al.[28] found statistically significant increase in activities of total plasma SOD ($p<0.003$, n = 32), plasma CAT ($p<0.0001$, n = 32) in septic patients when compared with healthy adult controls (n = 7). Further, they reported that within the group of septic patients, plasma SOD ($p<0.005$) and plasma CAT ($p<0.009$) (but not for RBC determination) when survivors (n= 15) were compared with non survivors (n=17). In their study, the most striking findings were that plasma total SOD value of >10 kU/L were found in 7 of 21 (30%) patients who couldn't survive their sepsis and that these values didn't overlap with any surviving patients or controls. However, while high total plasma SOD activity appears to have same potential as a prognostic indicator.

Seema et. al.[29] found an increase in xanthine oxidase, SOD and GPx activity in neonates with sepsis as compared to controls ($p<0.05$), suggesting increased production of ROS in this population. Further, they reported that α-TNF and free radicals have been implicated in the pathogenesis of neonatal septicemia and its complications.

Batra et. al. [30] found significantly elevated levels of serum XO, CPK, SOD and GPx activity and MDA in the neonates with sepsis when compared with controls. This study suggests increased production of ROS in neonates with sepsis, as evidenced 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 production.

Kapoor K et. al.[31] reported significantly increased activities of SOD, CAT and MDA ($p<0.05$) levels and depressed levels of UA ($p<0.001$) in babies with late-onset sepsis as compared to controls.

The most reasonable explanation of the increased serum activity of SOD and CAT in sepsis is that these increases are a result of tissue damage, as is true in several other clinical conditions. Secondly when ROS and RNS levels are increased in septic patients there would have been more production of SOD and CAT, so as to scavenge these free radicals.

Vitamin C

We found mean serum Vitamin C levels were significantly decreased in septic patients as compared to control subjects. Our results are supported by Karel Tyml et. al.[32],Borrelli et. al.[33]

Karel Tyml et. al. [32] recently showed in the mouse model of sepsis that an early intravenous injection of ascorbate bolus (i.e., at the onset of sepsis) prevents the development of platelet adhesion and plugging of capillaries at six hours of sepsis. Furthermore, ascorbate bolus injection delayed to six hours reverses this adhesion and plugging at seven hours of sepsis and that this beneficial effect of delayed ascorbate lasts at least 12 hours post-injection. In a mouse model, they showed that the early ascorbate prevents the increase in the plasma protein carbonyl (a measure of oxidative stress) and nitrate/ nitrite levels, the increase in iNOS protein expression in skeletal muscle, and the reduction in arteriolar contractility to norepinephrine and to

angiotensin II.

Borrelli et. al.[33] documented that plasma vitamin C was significantly decreased in ICU patients who developed multiple organ failure. Galley et al [34] reported increased redox reactive iron concentrations in patients with sepsis or septic shock, coupled with lowered plasma levels of Vitamin-C and elevated lipid peroxides.

The Present study showed a gradual decrease in vitamin C level in septic patients. The increase in ROS/RNS levels in septic patients contributes to oxidative stress. Oxidative stress is compensated by the usage of antioxidants like vitamin C. Therefore, there is a decrease in the level of vitamin C in septic patients.

Conclusion

Our study suggests infection and inflammation in septic patients leads to the production of pro inflammatory cytokines, ROS and RNS. This lead to imbalance in the inflammatory network and initiates oxidative stress-induced lipid peroxidation of membrane lipids, which is mainly responsible for cellular or organs injury that's confirmed by elevated levels of SOD and CAT.

Therefore these pro and anti inflammatory and oxidative stress markers are definitely used as a marker for SIRS and to assess progression and severity of the sepsis. Such immunomodulating agents may be therapeutically targeted to improve the clinical outcome of sepsis. These results showed the development of potential anticytokine and immunomodulating treatment strategies could improve the clinical outcome of sepsis patients

References

1. Bone R C, Balk R A, Cerra F B,et. al. J.Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis.The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine Chest, 1992;101(6):1644–1655.
2. Angus D C, Linde-Zwirble W T, Lidicker J, et. al. Epidemiology of severe sepsis in theUnited States: analysis of incidence, outcome, and associated costs of care.Critical Care Medicine, 2001; 29(7):1303–1310.
3. Martin G S , Mannino D M, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. The New England Journal of Medicine 2003;348(16):1546–1554
4. Dombrovskiy V Y, Martin A A, Sunderram J, Paz H L. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003.Critical Care Medicine .2007;35(5):1244–1250
5. Macarthur H, Westfall T C, Riley D P, et. al. Inactivation of catecholamines by superoxide gives new insights on the pathogenesis of septic shock. Proc Natl Acad Sci U S A. 2000; 97(17):9753–8
6. Salvemini D, Cuzzocrea S. Oxidative stress in septic shock and disseminated intravascular coagulation . Free RadicBiol Med. 2002;33:1173-1185
7. Ritter C, Andrades M E, Reinke A, et. al. Treatment with N-acetylcysteine plus deferoxamine protects rats against oxidative stress and improves survival in sepsis. Crit Care Med. 2004;32(2):342–9.
8. Sikora J P, Chlebna-Sokół D, Krzyzańska-Oberbek A. Proinflammatory cytokines (IL-6, IL-8), cytokine inhibitors (IL-6sR, sTNFRII) and anti-inflammatory cytokines (IL-10, IL-13) in the pathogenesis of sepsis in newborns and infants. Arch Immunol Ther Exp (Warsz). 2001; 49(5):399–404.
9. Satoh's K. Serum lipid peroxide in cerebrovascular disorder determined by a new colorimetric method. Clin Chim Acta. 1978,90:37–43
10. Najwa KC and Nabil WW. Determination of inorganic nitrate in serum and urine by kinetic cadmium reduction method. Clinical Chemistry. 1900; 36(8):1440–1443
11. Markland S and Marklund G. Involvement of the superoxide anion radical in acute oxidation of pyrogallol as a convenient assay for superoxide dismutase. Eur J Biochem.1974;4:469–474
12. Sinha K A. Colorimetric assay of catalase.Anul Biochem. 1972;47:389–394
13. Omaye S T, Turnbull J D and Sauberlich H E. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol. 1979;62:3–11
14. A M S, C R P Latex L; Quantitative turbidimetric latex assay for the measurement of C - reactive protein in human serum.
15. Gogos C A, Drosou E, Bassaris H P, Skoutelis A.. Pro- versus Anti-inflammatory Cytokine Profile in Patients with Severe Sepsis:A Marker for Prognosis and Future Therapeutic Options The Journal of Infectious Diseases 2000;181:176–80
16. Harbarth S, Holeckova K, Froidevaux C, et. al. Diagnostic Value of Procalcitonin, Interleukin-6, and Interleukin-8 in Critically Ill Patients Admitted with Suspected Sepsis. Am J Respir Crit Care Med 2001; 164(3):396–402
17. HiraoY, Kanda T, AsoY, et. al. Interleukin-8—An Early Marker for Bacterial Infection. Laboratory Medicine 2000;31(1):39–44
18. Lorente L, Martín M M, Abreu-Gonzalez P and Domínguez-Rodríguez A. Prognostic Value of Malondialdehyde Serum Levels in Severe Sepsis: A

Multicenter Study. PLoS ONE. 2013; 8(1):1371

19. Takeda K, Shimada Y, Amano M, et. al. Plasma lipid peroxides and alpha-tocopherol in critically ill patients. Crit Care Med. 1984;12:957–9
20. Ogilvie AC, Groeneveld AB, Straub JP, Thijs LG. Plasma lipid peroxides and antioxidants in human septic shock. Intens Care Med.1991;17:40-44
21. Mühl D, Woth G, Drenkovics L, et. al. Comparison of oxidative stress & leukocyte activation in patients with severe sepsis & burn injury. Indian J Med Res. 2011;134(1):69–78
22. Andrades M E, Cristiane R, Dal-Pizzol F, The role of free radicals in sepsis development, Frontiers in Bioscience. 2009;E1,277–287
23. Ochoa J B, Udekwu A O, Billiar T R, et. al. Nitrogen oxide levels in patients after trauma and during sepsis. AIIII Sllrg.1991; 214:621–626
24. Moncada S, PaImer R M and Higgs E A. Nitric Oxide: Physiology, Pathophysiology, and Pharmacology. Pharmacol Rev. 1991;43:109•142
25. Titheradge M A. Nitric oxide in septic shock. Biochim Biophys Acta. 1999;1411:437–455
26. Jennyfer Bultinck, Patrick Sips, Luc Vakaet, Peter Brouckaert, and Anje Cauwels. Systemic NO production during(septic) shock depends on parenchymal and not on hematopoietic cells: in vivo iNOS expression pattern in (septic) shock. The FASEB Journal. 2006;20:1620
27. Avontuur JA, Bruining HA, Ince C. Nitric oxide causes dysftmction of coronary autoregulation in endotoxemic rats. Cardiovasc Res. 1997;35:368–376
28. Ann Warner, Ana Bencosme, Daniel Healy and Catherine Verme. Prognostic Role of Antioxidant Enzymes in Sepsis: Preliminary Assessment.Clin Chem. 1995;41(6):867–871
29. Seema, Kumar R M and al R N, Tandon A, Randhawa V S, Mehta G, Batra S, Ray G N Kapoor A K. Serum TNF-alpha and free radical scavengers in neonatal septicemia. Indian J Pediatr. 1999;66(4):511–6
30. Batra S, Kumar R, Seema, Kapoor A K and Ray G. Alterations in antioxidant status during neonatal sepsis. Ann Trop Paediatr. 2000;20:27–33
31. Kapoor K, Basu S, Das B K and Bhatia B D. Lipid peroxidation and antioxidants in neonatal septicemia. J Trop Pediatr. 2006;52:372–5
32. KarelTyml. Critical Role for Oxidative Stress, Platelets, and Coagulation in Capillary Blood Flow Impairment in Sepsis. Microcirculation. 2011; 18(2): 152–162
33. Takeda K, Shimada Y, Amano M, et. al. Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. Crit CareMed, 1996;24:392–7
34. Galley H F, Howdle P D, Walker B E and Webster N R. The effects of intravenous antioxidants in patients with septic shock. Free Radic Biol Med, 1997;23:768–74.