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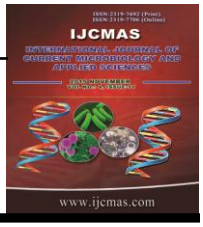

Original Research Article

Evaluation of Soluble E-Selectin and Total Antioxidant Capacity as Prognostic Biomarkers of Sepsis in Children

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A B S T R A C T

Sepsis is a serious clinical syndrome. Early diagnosis and predicting its prognosis can aid in prompt management. Soluble isoforms of adhesion molecules like soluble E-selectin can be used as diagnostic marker. On the other hand total antioxidant capacity measurement is thought to have value in diagnosis of this condition. The aim of the present study was to asses the relationship between serum level of total antioxidant capacity and soluble E-selectin (sE-selectin) to outcome of sepsis in children below 5 years affected with sepsis. The study included 111 child diagnosed by clinical criteria and by blood culture to have sepsis. In addition, thirty five healthy children were included as control. Serum total antioxidant capacity and sE-selectin was measured at end point of the study after 30 days. The mortality rate among patients was 31.5%. Total antioxidant capacity, sE-selectin and CRP levels were significantly higher in patients than control (P=0.0001). There was significantly increase in TAC level among non survival compared to survival (P=0.003) while sE-selectin was non significantly higher in survival than non survival (P=0.4). Receiver operating curve was done to detect a provisional cut off of serum TAC that may help in prediction of mortality. It revealed that a cut of 12.5 nmol/L has a validity of 74% with 95% confidence interval (64–86). Cox regression analysis of TAC are association with about 60% cumulative survival of cases more than 30 days hazard ratio =1.109 with 95% confidence interval (1.053–1.169). Cox regression revealed non significant role of CRP, E selectin and age The present study highlights the use of biomarkers as prognostic tools in children below five years with sepsis. Both sE-selectin and total antioxidant capacity was increased in children with sepsis. However, the most distinguished finding of the present study that total antioxidant capacity is increased significantly in sepsis and even could be used as a prognostic marker for mortality.

Keywords

TAC,
sE-Selectin,
Mortality,
Children

Introduction

Sepsis is a common and serious condition. It is defined as systemic inflammatory response syndrome with the presence of a pathogen. Sepsis is claimed to be

responsible as a major cause of death in children below 5 years (1).

Sepsis syndrome is a systemic immune response to invading pathogens. The pathogenesis of sepsis is mainly attributed to the adherence of leukocytes with vascular endothelium and this leads to the progrssion of inflammatory cascade. There are five adhesion molecules that differed according to their biochemical structures and functions. Three adhesion molecules belong to selectin superfamily and serve as leukocytes rolling molecules named as E-selectin, L- selectin and P-selectin and two intracellular adhesion molecules namely intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1)) belong to the immunoglobin domain superfamily cell adhesion molecules which are important for firm adhesion and transendothelial migration (2). The adhesions molecules at surfaces of cells shed out to the circulation as soluble isoforms that can be used as a marker for ongoing inflammatory procedures. These isoforms are thought to play a regulatory role in reducing inflammation by slowing the adhesion of leucocytes to the endothelium thus protect the patient from prononunced uncontrolled leukocytes adhesions (3,4). The levels of these adhesion molecules vary according to age of patients and the severity of underlying inflammatory conditions. However, there are scarce studies about the relation between level of adhesion molecules and the outcome of sepsis (5), especially in children.

Other contributing factor in the pathogenesis of sepsis is the production of reactive oxygen and nitrogen species to the circulation (6-10). To oppose the side effects of overproduction of reactive oxygen, there is over production of antioxidants. When the infection continues, oxidative stress leads to significant damage to cells and mitochondria components including lipids, protein and carbohydrate. Later on, mitochondrial disruption results in failure in energy chain with organ dysfunction and death (11-13). The balance between both oxidant and antioxidant system can be measured by total antioxidant capacity (TAC) in serum giving the mirror image about patient antioxidant status (10).

Previous reports were carried out for measurement of TAC to know its role in severe conditions of illness and sepsis (14-23). However, scarce reports have described the putative role of TAC associated with mortality in sepsis in pediatric patients (24).

The aim of the present study was to asses the relationship between serum levels of total antioxidant capacity and soluble E-selectin (sE-selectin) the outcome of sepsis in children below 5years affected with sepsis.

Material and Methods

The study was carried out at Mansoura University children hospital during the period from January 2015 to September 2015. It is cross sectional study. Children below 5 years defined having sepsis clinically and proved by positive blood culture were included in the study. Clinical sepsis was identified by the presence of 3 or more of the clinical signs derived from sepsis score including temperature instability (hypothermia, hyperthermia); respiratory distress, cardiovascular signs like bradycardia, and neurologic manifestations like hypotonia (25,26). The children were recruited into the study at the time of evaluation for suspected clinical sepsis before the start of antibiotic therapy. In addition, healthy thirty five children with matched age and sex were included as

control. Informed written consent was obtained from the parents of each child. The study was approved by ethical committee of Mansoura faculty of medicine, Egypt.

A full laboratory sepsis screen, which included cultures from blood and C-reactive protein (CRP) measurement by turbidimetry, was performed. Aerobic bacteria were identified using the Microscan system (Beckman). Parenteral antibiotics were started immediately after the samples for the infection screen had been obtained.

The following variables were recorded for each patient: sex, age, underlying conditions, microorganism responsible and empiric antimicrobial treatment.

End point

End point was 30-day mortality.

Blood samples

Six ml Blood samples from 111 children were collected within 2 hours of the diagnosis of severe sepsis. One ml of blood was inoculated to Bact/Alert pediatric blood culture bottle for microbiological culture. Isolated bacterial pathogen were further sub cultured for complete microbiological identification and antimicrobial susceptibility determination by automated Microscan system.

Five millimeter blood sample from each patient was allowed to clot and serum was separated and serum was stored in aliquot at − 80°C for measurement of total antioxidant capacity and sE-selectin assays.

Serum TAC level analysis (BioVision, Inc., San Francisco, USA)

TAC Assay Kit, which can measure either the combination of both small molecule antioxidants and proteins or small molecules alone in the presence of protein Mask. Cu^{2+} ion is converted to Cu+ by both small molecule and protein. The Protein Mask prevents Cu^{2+} reduction by protein, enabling the analysis of only the small molecule antioxidants. The reduced Cu+ ion is chelated with a colorimetric probe giving a broad absorbance peak around 570 nm, proportional to the total antioxidant capacity.

Serum Detection of sE-selectin

sE-selectin concentrations of sE-selectin were determined using a E-selectin enzyme-linked immunosorbent assay kit (Diaclone Research, France). This technique uses a monoclonal antibody that is specific for E-selectin, which is precoated onto the wells of microtiter strips.

Statistical Analysis

Statistical analysis was performed using SPSS 16. Continuous variables are reported as mean± SD ranges. Categorical variables are reported as frequencies and percentages. Comparisons of continuous variables between groups were carried out using Mann-Whitney U test.

Comparisons between groups for categorical variables were carried out with $\chi 2$ test and the P value is a global P value. We plotted a receiver operating characteristic curve using survival at 30 days as classification variable and TAC serum level as prognostic variable. Cox regression analysis was applied to determine the independent contribution of TAC serum levels on the prediction of 30-days mortality.

Results and Discussion

The study included 111 children with clinically suspected sepsis and confirmed by positive blood culture. Thirty five children with cross age and sex were included as healthy control. The mean age of patients was 33±3.5 months. They were 59 males and 52 females. The isolated bacterial culture was Gram positive cocci in 72.1% of patients (mainly *Staphylococcus aereus* in 50 cases and *Staphlococcus* coagulase negative in 30 cases, data not shown). The mortality rate among patients was 31.5%

Total antioxidant capacity, sE-selectin and CRP levels were significantly higher in patients than control(P=0.0001), table 1.

Table (2) summarized the findings among survival and non survival. There was significantly increase in TAC level among non survival compared to survival (P=0.003) while sEselectin was non significantly higher in survival than non survival (P=0.4), table 2

Receiver operating curve was done to detect a provional cut off of serum TAC that may help in prediction of mortality. It revealed that a cut of 12.5 has a validity of 74% with 95% confidence interval (64-86), figure 1.

Cox regression analysis of TAC are association with about 60% cumulative survival of cases more than 30 days hazard ratio =1.109 with 95% confidence interval (1.053-1.169). Cox regression revealed non significant role of CRP, E selectin and age, figure 2.

Sepsis is a model for systemic inflammatory response in a trial to eliminate the infection. It results in activation of both haemostatic and immune system leading to severe inflammation involving vascular thrombosis and microcirculatory failure leading to organ failure (27-30).Unfortunately even with the extensive studies of pathogenesis of septic syndrome mortality rate due to this condition is still high. In the present study the mortality rate was (31.5%) similar to previous reported rate between 20-30% (31, 32).Gram positive cocci compromised of all isolated organisms with *Staphylococcus aereus* being the commonest (33).

Several adhesion molecules were studied as potential biomarkers for early diagnosis of sepsis and as prognostic biomarkers (34). Among the studied biomarkers, sE-Selectin represent an imminent biomarker due to its early role as an adhesion molecule for the activated neutrophil to endothelium a phenomena implicated in pathogenesis of sepsis and organ failure. Soluble form of E-selectin circulates in low level in healthy subjects and increase dramatically in various systemic inflammatory procedures (35-39).

In the present study sE-selectin level was significantly higher in patients than control (P=0.0001). However, there was non significant higher level of sE-selectin among survival compared to non survival (P=0.4). Similar results were reported in previous studies (25, 40, 41) with significantly increased levels of sE-Selectin 1 week after stress. Even with longitudinal study within 7 days duration, sE-Selectin was higher in patients with sepsis, especially among survivors (42). The non significant increase in the present study can be attributed to extended time of our study after 30 days survival measurement or the difference in the age of studied patients in our study.

Another squeal of protective systemic immune response in sepsis is the production of massive amount of reactive oxygen species from activated leukocytes. Reactive oxygen species contribute to bacterial killing

however it has potential hazards to the host (43, 44). Extensive production of reactive oxygen species results in apoptotic cells death and implicated in multiple organ failure following sepsis (45). To balance these effects total antioxidant capacity increases in sepsis. These clarify the significant increase in TAC in patients compared with control in the present study. Moreover, there was significant increase in TAC in non survivors compared to survivors (P=0.003), similar finding reported by Abreu-González et al., 2015 (46) in critically ill adults. It is clear that higher TAC levels in non survivors is a consequent event to the increase of antioxidant capacity during the early phase of sepsis to compensate the increase production of reactive oxygen species (47).

Table.1.Demographic and Laboratory Findings of Patients and Control

	Patients (n=111)	Control (n=35)
Sex Male Female	 59 53.2% 52 46.8%	 19 (54.3%) 16 (45.7%)
Age months	 33± 3.5	 32±2.8
Isolated Bacteria Gram Positive Gram Negative	 80 72.1% 31 27.9%	
Death alive	35 31.5% 76 68.5%	
Previous antibiotics therapy	111 100%	
TAC (nmol/L)	13.7± 6.8	1.1± 0.1 P= 0.0001
Eselectin (ng/ml)	27.5± 3.75	2.7±1.5 P=0.0001
CRP Mean± SD Median Minimum Maximum	 12 6 96	 3.4±1.2 P=0.0001

Table.2. Comparison between survival and non survival patients

	Survival (n=76)	Non survival (n=35)	P
Age (months)	47.7± 41.4	36.6± 16.5	P=0.2
TAC mmol/L	11.5± 4.7	18±8.1	P=0.003
E-selectin ng/ml	31.1± 0.2	29.1± 1.5	P=0.4
CRP(mg/dl)	17.6± 12.3	23.6± 18.6	P=0.5

Figure.1. Receiver operating curve for TAC

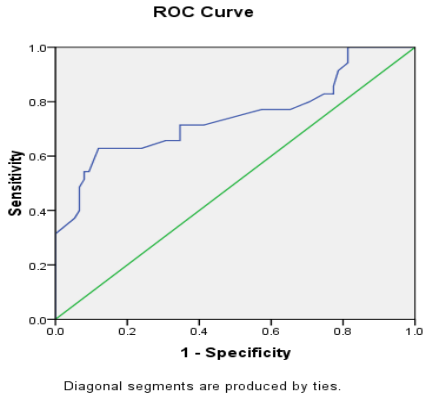
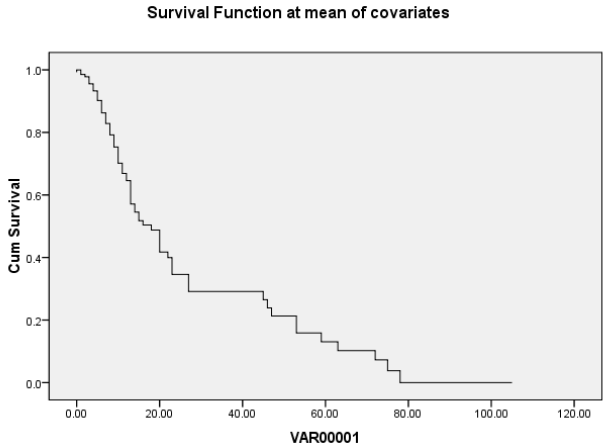


Figure.2. Cox regression analysis curve for for TAC



The distinguished finding in the present study is the capacity of TAC level to predict fatal outcome of sepsis in children below five. Cox regression analysis of TAC are association with about 60% cumulative survival of cases more than 30 days hazard ratio =1.109 with 95% confidence interval (1.053-1.169). Moreover, Receiver operating curve was done to detect a cut off of serum TAC that may help in prediction of mortality. It revealed that a cut of 12.5 has a validity of 74% with 95% confidence interval (64-86). To our knowledge this is the first study that represented a cut off value with reasonable validity for TAC that can be used as a prognostic biomarker in children below five with severe sepsis.

Taken together, these data suggest that the increased antioxidant capacity observed in non survivors is a host response to severely propagating oxidative stress.

The present study highlights the use of biomarkers as prognostic tools in children below five with sepsis. Both sE-selectin and total antioxidant capacity was increased in children with sepsis. However, the most distinguished finding of the present study that total antioxidant capacity is increased significantly in sepsis and even could be used as a prognostic marker for mortality.

Reference

1.Plunkett A. Sepsis in children. BMJ 2015; 350-55

2.Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 2007; 7:678–689

3.Garton KJ, Gough PJ, Raines EW. Emerging roles for ectodomain shedding in the regulation of inflammatory responses. J Leukoc Biol. 2006;79:1105–1116. doi: 10.1189/ jlb.0106038.

4.Long C, Wang Y, Herrera AH, Horiuchi K, Walcheck B. In vivo role of leukocyte ADAM17 in the inflammatory and host responses during E. coli-mediated peritonitis. J Leukoc Biol. 2010;87:1097–1101.

5.Briassoulis G, Papassotiriou I, Mavrikiou M, Lazaropoulou C, Margeli A. Longitudinal course and clinical significance of TGF-beta1, sL- and sE-Selectins and sICAM-1 levels during severe acute stress in children. Clin Biochem. 2007;40:299–304.

6.Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006;34: 344–53.

7.Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29:1303–10.

8.Dare AJ, Phillips ARJ, Hickey AJR, Mittal A, Loveday B, Thompson N, et al. A systematic review of experimental treatments for mitochondrial dysfunction in sepsis and multiple organ dysfunction syndrome. Free Radic Biol Med 2009;47:1517–25.

9.Andrades ME, Ritter C, Dal-Pizzol F. The role of free radicals in sepsis development. Front Biosci 2009;1:277–87.

10.Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med 2000;29:1106–14.

11.Galley HF. Oxidative stress and mitochondrial dysfunction in sepsis. Br J Anaesth 2011;107:57–64.

12.Brealey D, Singer M. Mitochondrial dysfunction in sepsis. Curr Infect Dis Rep 2003;5: 365–71.

13.Crouser ED. Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. Mitochondrion 2004;4:729–41.

14-Cheng CH, Huang SC, Chiang TY, Wong Y, Huang YC. Higher plasma pyridoxal phosphate is associated with increased antioxidant enzyme activities in critically ill surgical patients. Biomed Res Int 2013;2013:572081.

15.Alonso de Vega JM, Díaz J, Serrano E, Carbonell LF. Oxidative stress in critically ill patients with systemic inflammatory response syndrome. Crit Care Med 2002;30: 1782–6.

16.Dasgupta A, Malhotra D, Levy H, Marcadis D, Blackwell W, Johnston D. Decreased total antioxidant capacity but normal lipid hydroperoxide concentrations in sera of critically ill patients. Life Sci 1997;60:335–40.

17.MacKinnon KL, Molnar Z, Lowe D, Watson ID, Shearer E. Measures of total free rad- ical activity in critically ill patients. Clin Biochem 1999;32:263–8.

18.Tsai K, Hsu TG, Kong CW, Lin K, Lu F. Is the endogenous peroxyl-radical scavenging capacity of plasma protective in systemic inflammatorydisorders in humans? Free Radic Biol Med 2000;28:926–33.

19.Karapetsa M, Pitsika M, Goutzourelas N, Stagos D, Tousia Becker A, Zakynthinos E. Oxidative status in ICU patients with septic shock. Food Chem Toxicol 2013. http://dx.doi.org/10.1016/j.fct.2013.03.026 [pii: S0278-6915(13)00209-3, Epub ahead of print].

20.Doise JM, Aho LS, Quenot JP, Guilland JC, Zeller M, Vergely C, et al. Plasma antioxi- dant status in septic critically ill patients: a decrease over time. Fundam Clin Pharmacol 2008;22:203–9.

21.Chuang CC, Shiesh SC, Chi CH, Tu YF, Hor LI, Shieh CC, et al. Serum total antioxidant capacity reflects severity of illness in patients with severe sepsis. Crit Care 2006;10: R36.

22.Pascual C, Karzai W, Meier-Hellmann A, Oberhoffer M, Horn A, Bredle D, et al. Total plasma antioxidant capacity is not always decreased in sepsis. Crit Care Med 1998; 26:705–9.

23.Cowley HC, Bacon PJ, Goode HF, Webster NR, Jones JG, Menon DK. Plasma antioxi- dant potential in severe sepsis: a comparison of survivors and nonsurvivors. Crit Care Med 1996;24:1179–83.

24.von Dessauer B, Bongain J, Molina V, Quilodrán J, Castillo R, Rodrigo R. Oxidative stress as a novel target in pediatric sepsis management. Crit Care. 2011;26(1):103.e1-7.

25.Zaki Mel-S, el-Sayed H. Evaluation of microbiologic and hematologic parameters and E-selectin as early predictors for outcome of neonatal sepsis. Arch Pathol Lab Med. 2009 Aug;133(8):1291-6

26.Tollner, U. Early diagnosis of septicaemia in the newborn: clinical studies and sepsis score. Eur J Pediatr 1982. 138 4:331–337

27.Maniatis NA, Kotanidou A, Catravas JD, Orfanos SE. Endothelial pathomechanisms in acute lung injury. Vascul Pharmacol 2008;49(4–6):119–33.

28.Schouten M, Wiersinga WJ, Levi M, van der Poll T. Inflammation, endothelium and coagulation in sepsis. J Leukoc Biol 2008; 83(3):536–45.

29.American College of Chest Physicians/Society of Critical Care MedicineConsensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992; 20(6): 864–74.

30.Orfanos SE, Mavrommati I, Korovesi I, Roussos C. Pulmonary endothelium in acute lung injury: from basic science to the critically ill. Intensive Care Med 2004;30(9):1702–14.

31.Hatherill M, Tibby SM, Turner C, et al: Procalcitonin and cytokine levels: Relationship to organ failure and mortality in pediatric septic shock. Crit Care Med 2000; 28: 2591–2594

32.Ceneviva G, Paschall JA, Maffei F, et al: Hemodynamic support in fluid-refractory pediatric septic shock. Pediatrics 1998; 102:e19

33.Oda, K., Matsuo, Y., Nagai, K., Tsumura, N., Sakata, Y. and Kato, H. (2000), Sepsis in children. Pediatrics International, 42: 528–533.

34.Xing K, Murthy S, Liles WC, Singh JM. Clinical utility of biomarkers of endothelial activation in sepsis – a systematic review. Crit Care 2012;16(1):R7.

35.Sessler CN, Windsor AC, Schwartz M, Watson L, Fisher BJ, Sugerman HJ, et al. Circulating ICAM-1 is increased in septic shock. Am J Respir Crit Care Med 1995;151(5):1420–7.

36.Cowley HC, Heney D, Gearing AJ, Hemingway I, Webster NR. Increased circulating adhesion molecule concentrations in patients with the

systemic inflammatory response syndrome: a prospective cohort study. Crit Care Med 1994;22(4):651–7.

37.Boldt J, Wollbruck M, Kuhn D, Linke LC, Hempelmann G. Do plasma levels of circulating soluble adhesion molecules differ between surviving and nonsurviving critically ill patients? Chest 1995;107(3):787–92.

38.Boldt J, Muller M, Kuhn D, Linke LC, Hempelmann G. Circulating adhesion molecules in the critically ill: a comparison between trauma and sepsis patients. Intensive Care Med 1996;22(2):122–8.

39.Knapp S, Thalhammer F, Locker GJ, Laczika K, Hollenstein U, Frass M, et al. Prognostic value of MIP-1 alpha, TGF-beta 2, sELAM-1, and sVCAM-1 in patients with gram-positive sepsis. Clin Immunol Immunopathol 1998;87(2):139–44.

40.Stengel D, Orth M, Tauber R, Sehouli J, Hentsch S, Thielemann HK, et al. Shed L-selectin (sCD62L) load in trauma patients. J Surg Res 2001;99: 321–7.

41.Vassiliou A G. , Mastora Z , Orfanos S E., Jahaj E, Maniatis N A., Koutsoukou A, Armaganidis A, Kotanidou A. Elevated biomarkers of endothelial dysfunction/activation at ICU admission are associated with sepsis development, Cytokine 69 (2014) 240–247

42.Briassoulis G, Papassotiriou I, Mavrikiou M, Lazaropoulou C, Margeli A Longitudinal course and clinical significance of TGF-1, sL- and sE-Selectins and sICAM-1 levels during severe acute stress in children. Clinical Biochemistry 40 (2007) 299–304

43.Babior BM. The respiratory burst of phagocytes. *J Clin Invest* 1984; *73*: 599-601.

44.Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med*1989; *320*: 365-76

45.Cherian S, Jameson S, Rajarajeswari C, Helena V, Lakshmi Latha Anu Rekha M.R.,et al., Oxidative stress in sepsis in children Indian J Med Res 125, 2007, 143-148

46.L Lorente,, M M. Martín, T Almeida, Abreu-González P, et al., Association between serum total antioxidant capacity and mortality in severe septic patients, Journal of Critical Care 30 (2015) 217.e7–217.e12

47.MacKinnon KL, Molnar Z, Lowe D, Watson ID, Shearer E. Measures of total free radical activity in critically ill patients. Clin Biochem 1999;32:263–8.