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Oxidative stress in sepsis in children

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***Background & objectives*: Information on oxidative damage during spesis in children is not available, we undertook this study to assess the levels of certain antioxidants in blood of children with sepsis.**

***Methods*: Study group had 38 children with sepsis (<5 yr) and 39 age-and sex-matched controls admitted to a tertiary care hospital. Red cell glutathione (GSH), superoxide dismutase (SOD) and thiobarbituric acid reactive substance (TBARS) and plasma vitamin C were estimated by standard techniques.**

***Results*: There was no significant change in erythrocyte GSH, SOD and TBARS levels in sepsis when compared to controls. This may be due to the adaptive response of the body to combat the oxidative stress. 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.**

***Interpretation & conclusion*: Our findings show that children affected by sepsis probably adapt to the free radical toxicity induced by this condition. Further studies need to be done on a larger sample to confirm the findings.**

Key words Glutathione - lipid peroxidation - paediatric sepsis - superoxide dismutase - vitamin C

Sepsis is known to be a common cause of morbidity and mortality in critically ill patients[1,2]. The inflammatory response to critical illness involves the activation of leukocytes and other inflammatory cells leading to a massive production of reactive oxygen species (ROS). ROS mediated oxidative stress has been implicated in apoptotic cell death and in turn, can be harmful to the patient when the endogenous antioxidant defense mechanisms are overwhelmed[3]. Alterations in the apoptotic programme may underlie the dysregulation of the inflammatory response that occurs during sepsis. It is now well documented that ROS is involved in the pathogenesis of

multiple organ failure following sepsis, often leading to death[4].

Information is largely not available on the oxidative damage in sepsis in children. Hence, in the present study an attempt was made to assess free radical toxicity following sepsis in children below the age of five years.

Material & Methods

A total of 38 children <5 yr of age with sepsis, selected consecutively during May to December 2002 were enrolled in this study, admitted to the paediatric wards of Kasturba Hospital, Manipal. This is a tertiary care hospital, hence these patients were referred cases. They were divided into two groups based on the age as follows:

Group 1: Children between 1 month - 1 yr (n=13, 7 males, 6 females).
Group 2: Children between 1 yr 1 month - 5 yr (n=25,13 males, 12 females).

Age and sex-matched controls (n=39) were children who were posted for elective surgery for umbilical hernia, hydrocele and phimosis and had no clinical evidence of infection. The nutritional status of the children, both controls and patients was assessed according to the anthropometric parameters enlisted by the Indian Academy of Pediatrics[5]. The inclusion criterion followed to diagnose sepsis was according to the Society of Critical Care Medicine Consensus Committee[6] taking into consideration body temperature, heart rate, respiratory rate, WBC count, *etc*. On the other hand, children with chronic diseases like nephrotic syndrome malignancies/chemotherapy, collagen vascular disease, congenital heart disease, neurodegenerative disease, chronic renal failure,chronic liver disease, inborn errors of metabolism, and other immunocompromised states like children infected with HIV and those on long course of steroids were excluded from the study (4 out of a total of 42 initially included in the study). The primary diagnosis in about 80 per cent of the children in both the groups was pneumonia whereas the rest were suffering from meningitis and gastroenteritis. All the patients needed intensive care unit facility of the hospital. Sepsis resulted in 15 and 8 per cent mortality in groups 1 and 2 respectively.

Sample collection and preparation: Approval of study protocol was obtained from the institutional ethical committee. Written consent was obtained from the parents of patients.

Blood (2 ml) was collected into EDTA bottles from both controls (preoperative) and patients at the time of confirmed diagnosis before starting treatment on admission to the hospital. The erythrocyte suspension was prepared according to the method of Beutler *et al*[7]. It was immediately centrifuged under refrigeration at 3000 x g for 10 min. Plasma and the buffy coat were carefully removed and the separated cells were washed thrice with cold saline phosphate buffer, *p*H 7.4 (sodium phosphate buffer containing 0.15 mol/l NaCl). The erythrocytes were then suspended in an equal volume of physiological saline. Appropriately diluted haemolysates were then prepared for the estimation of glutathione (GSH), superoxide dismutase (SOD) and thiobarbituric acid reactive substance (TBARS). The plasma was used for the estimation of vitamin C. GSH was estimated colorimetrically[8]. SOD was analyzed by the method of Beauchamp and Fridovich[9]. Plasma vitamin C was determined colorimetrically[10]. Lipid peroxidation products were quantified by the thiobarbituric acid method[11]. The haemoglobin content of the erythrocyte was estimated by the cyanmethhaemoglobin method[12].

Statistical analysis of data was carried out by the non-parametric unpaired Mann-Whitney U-test.

Results & Discussion

The haemoglobin level in both controls and patients exhibited no significant change. The erythrocytic GSH, SOD and TBARS levels showed no significant change in both the groups of patients (1 and 2) when compared to controls. The vitamin C levels did not show any change in group 1 (Tables I and II). However, in group 2 there was a significant decrease in vitamin C levels when compared to controls. Two children each in group 1 and 2 died, the cause of death being multiorgan failure. There was no significant difference between the findings in children who expired and those who survived.

We found no change in the erythrocytic antioxidants GSH and SOD as well as in the levels of the lipid peroxidation product, TBARS in this study.This was in contrast to earlier observations where increased serum SOD, xanthine oxidase, glutathione peroxidase (GP), malondialdehyde (MDA) and decreased uric acid and albumin have been reported in neonatal sepsis[13]. Moreover, a significant increase in serum tumour necrosis factor-alpha was shown to be accompanied by a significant increase in SOD and GP in newborns with sepsis[14]. Increased serum MDA and 4-hydroxyl alkenals have also been reported in septic newborns[15]. MDA is a reliable indicator of lipid

Table I. Comparison of levels of antioxidants and lipid peroxidation between group 1 patients and age matched controls

	SOD (Units/gHb)	GSH (mg/gHb)	Vitamin C (µmol/l)	TBARS (nmol/gHb)
Controls	2176.49	8.29	17.90	2.31
	(1824.47-3326.34)	(6.24-10.31)	(14.90-22.00)	(1.37-3.36)
	(n=13)	(n=12)	(n=12)	(n=12)
Cases	3030.97	10.51	18.70	3.23
	(2253.42-4297.36)	(6.00-28.26)	(12.60-21.70)	(1.60-4.50)
	(n=13)	(n=13)	(n=12)	(n=12)

Values are shown as median (inter quartile range)
SOD, superoxide dismutase; GSH, glutathione; TBARS, thiobarbituric acid reactive substance

Table II. Comparison of levels of antioxidants and lipid peroxidation between group 2 patients and age matched controls

	SOD (Units/gHb)	GSH (mg/gHb)	Vitamin C (µmol/l)	TBARS (nmol/gHb)
Controls	2156.01	7.92	13.80	5.32
	(1418.69-3572.16)	(5.07-12.68)	(11.50-20.30)	(2.94-8.12)
	(n=26)	(n=26)	(n=25)	(n=26)
Cases	2536.80	11.32	11.40*	3.59
	(1755.13-3800.60)	(7.21-17.96)	(7.40-15.30)	(1.83-6.40)
	(n=24)	(n=25)	(n=24)	(n=25)

Values are shown as median (inter quartile range)
*P<0.05 compared to controls

peroxidation as a result of free radical toxicity in biological materials. However, there are reports[16] where the plasma total radical trapping parameter (TRAP) decreased until death in systemic inflammatory response syndrome (SIRS) suggesting that there is no protective role of the endogenous peroxyl-radical scavenging capacity of plasma in SIRS in humans. A significant decrease in vitamin C levels in group 2 in this study may be due to active phagocytosis. Besides, it may participate in metal binding reactions, particularly those involving free iron and copper thus, protecting free radical injury and increasing survival[17]. Since all children were well nourished, this decrease may not be due to difference in dietary intake of vitamin C. Further, there are suggestions of including vitamin C in the treatment of sepsis due to its role as a modulator of immune function in such cases[18]. The prooxidant-antioxidant balance has functional relevance during critical illness including sepsis because it is involved in the pathogenesis of multiple organ failure[19,20] due to production of a wide range of reactive oxygen species (ROS). Activated phagocytes produce a number of products *viz.*, superoxide radical, hydrogen peroxide and hypochlorus acid that not only contribute to bacterial killing but are also potentially toxic to host tissues[21,22]. Human cells and extracellular fluids contain an equally wide range of antioxidants that have been well characterized in plasma, one of them being ascorbic acid[23,24]. Schorah *et al*[25] showed that the plasma concentrations of vitamin C were significantly decreased in critically ill patients and also that the antioxidant defenses could be considerably compromised in these very sick patients. This marked depletion of circulating concentrations of vitamin C may be because it is exhaustively consumed in sepsis to decrease lipid peroxidation and levels of hydrogen peroxide[26]. Further, dysregulation of the immuno-inflammatory response as seen in sepsis, may culminate in host cell and organ damage. The increase in immune system response may lead to increased release of cytokines which in turn may produce overwhelming amounts of ROS/reactive nitrogen species/free radicals leading to catastrophic tissue damage[27]. Therefore, equally overwhelming amounts of antioxidants are required in sustained concentration to both, prevent and combat this stress which otherwise could effect cellular function and regulation of gene expression[26,28]. However, children between the age of one month to one year showing no significant change in plasma vitamin C levels in the present study, probably adapt better to the oxidative stress in sepsis. It has also been suggested that vitamins C and E improve hepatic drug metabolizing dysfunction as indicated by abnormalities in cytochrome P 450 isoforms during sepsis, and this protection is mainly caused by decreased oxidant stress and lipid peroxidation[29]. Vitamin C acts primarily as an antioxidant at low doses whereas the pro-oxidant effects of vitamin C are observed at high doses in hepatic ischaemia and reperfusion[30]. Recently, vitamin C was reported to prevent microvascular dysfunction in septic animals[31].

In conclusion, children with sepsis did not exhibit antioxidant depletion or enhanced lipid peroxidation in our study. The fact that the children exhibited an efficient adaptive response as they were not malnourished may account for this observation. Further studies need to be done to see whether supplementation with antioxidants (vitamin C) improves the prognosis in such cases.

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