Study of Antioxidants' Enzymes Kinetics, Superoxide Dismutase, Glutathione Peroxidase and Catalase, in Neonatal Sepsis

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ABSTRACT

Context: sepsis is associated with severe oxidative stress. During sepsis, there are several potential sources of reactive oxygen species, including the respiratory burst associated with neutrophil activation.

Aim: We try to monitor the kinetics of the scavengers' antioxidant enzymes, erthrocyte superoxide dismutase (SOD), whole blood glutathione peroxidase (GPX) and serum catalase in the pathogenesis of neonatal sepsis and the effect of antibiotics therapy on their levels.

Study Design: This study was carried out on blood samples from children suffering from sepsis proved by positive blood culture in addition to 11 healthy children. Blood samples were subjected for determination of antioxidant enzyme activities including red blood cell superoxide dismutase (SOD), whole blood glutathione peroxidase (GPx) and serum catalase

Results; There was highly significant decrease in erythrocyte superoxide dismutase (RBC SOD) ($P<0.001$) and whole blood glutathione peroxidase (GPx) ($P=0.01$) at admission and after 48 hours of start of antibiotics therapy ($P<0.001$, for each) in patients compared with controls. There was highly significant increase in serum catalase level in patients at admission ($P<0.001$) and after 48 hours of antibiotics therapy compared with controls. The commonest isolated organism from blood culture was *Staphylococci species* (66.7%) with *Staphylococcus aureus* (40.0%) and *Staphylococci epidermidis* (26.7%). There was significant decrease of SOD ($P1=.0001$) and GPx ($P1=.049$) at start of sepsis associated with *Staphylococcus aureus* infection. After 48 hours from clinical response to antibiotics therapy, both SOD and GPx had significant decrease with all types of bacteria isolated.

Conclusion: Our study suggests that children with sepsis are susceptible to high oxidative stress which may play a role in the pathogenesis of sepsis. The administration of antibiotics therapy was not associated with the improvement of level of superoxide dismutase and glutathione peroxidase, so the utility of supplementation of antioxidant enzymes in neonates with septicemia needs further evaluation.

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INTRODUCTION

Sepsis is an old and intractable problem, the control of which has steadily worsened in recent years, despite many advances in clinical care (Dellinger, *et al.*, 1997). The exact reasons for this difficult situation are uncertain but there seems to be little doubt that a growing resistance by bacteria to antibiotics is a major contributor (Michel and Gutmann 1997).

In sepsis, there is convincing evidence of severe oxidative stress. During sepsis, there are several potential sources of reactive oxygen species, including mitochondrial respiratory electron transport chain, xanthine oxidase activation as a result of ischemia and reperfusion, the respiratory burst associated with neutrophil activation, and arachidonic acid metabolism. Activated neutrophils produce superoxide as a cytotoxic agent as part of the respiratory burst via the action of membrane-bound NADPH oxidase on molecular oxygen. Neutrophils also produce the free radical nitric oxide which can react with superoxide to produce peroxynitrite a powerful oxidant, which may decompose to form the hydroxyl / radical (OH.) (McDonald, *et al.*, 2003). Free radicals have been implicated in the pathogenesis of neonatal septicemia (Kapoor *et al.*, 2006).

During oxidative stress, damage mediated by reactive oxygen species can occur. Oxidation of DNA and proteins may take place, along with membrane damage, because of lipid peroxidation, leading to alterations in membrane permeability. This can ultimately lead to mitochondrial damage, with release of cytochrome, activation of caspases and apoptosis (McDonald *et al.*, 2003).

Under normal physiological conditions, a homeostatic balance exists between the formation of reactive oxidizing / oxygen species and their removal by endogenous antioxidant scavenger's compounds. Antioxidant is central to the red ox balance in the human body. They do not act in isolation, but synergistically (Gutteridge and Mitchell 1999).

Primary antioxidants prevent oxygen radical formation, whether by removing free radical precursors or by inhibiting catalysts, e. g. glutathione peroxidase and catalase. Secondary antioxidants react with reactive oxygen species which have already been formed, either to remove or inhibit them, e. g. vitamins C and E.

Endogenous antioxidant defenses exist at a number of locations, namely intracellular, on the cell membrane and extracellularly (Gutteridge and Mitchell 1999). Few reports discuss the kinetics of scavengers' enzymes pathways during sepsis and no reports to our knowledge discuss the effect of antibiotics therapy on such pathways.

In this work we try to monitor the kinetics of the scavengers' antioxidant enzymes, erthrocyte superoxide dismutase (SOD), whole blood glutathione peroxidase (GPX) and serum catalase in neonatal sepsis and the effect of antibiotics therapy on their levels.

PATIENTS AND METHODS

Patients

This study was carried out on 30 neonates suffering from sepsis in addition to 11 healthy neonates of matched age and sex. Clinical sepsis was defined as the presence of three or more of the following categories of clinical signs derived from a validated sepsis score: (a) temperature instability (hypothermia, hyperthermia); (b) respiratory (grunting, intercostal retractions, apnea, tachypnea, cyanosis); (c) cardiovascular (bradycardia, tachycardia, poor perfusion, hypotension);(d) neurologic (hypotonia, lethargy, seizures); (e) gastro intestinal (feeding intolerance, abdominal distension)(5). Parenteral antibiotics were started immediately after the infection screen and the blood samples had been taken. The children were recruited into the study at the time of evaluation for suspected clinical sepsis before start of antibiotic therapy and after 48 hours of antibiotics therapy. Informed written consent was taken from the parents of each child. The study was approved by ethical committee of Mansoura University.

METHODS

A full laboratory sepsis screen was performed which included cerebrospinal fluid analysis, and cultures from blood, urine. Blood culture was performed by the Becton Dickinson System (BACTEC 9050) using BD BACTE PEDS plus TM/F culture vials (soybean-casein digest broth with resins).The positive blood culture bottles were processed and the bacterial isolates were identified using Walkway system. *Staphylococci epidermidis* was considered the pathogenic bacteria after its isolation from two culture sets of the patient.

Two blood samples were collected from each patient; the first was withdrawn at admission to the hospital and the second after 48 hours after administration antibiotics. Blood samples were subjected to laboratory for determination of antioxidant enzyme activities of red blood cell superoxide dismutase (SOD), whole blood glutathione peroxidase (GPx) and serum catalase.

Assay of erythrocyte superoxide dismutase activity (SOD). The activity of SOD was measured according to the method of Nishikimi *et al.,* (1972).This assay depends on ability of the enzyme to inhibit phenazine methosulphate

(PMS) mediated reduction of nitroblue tetrazolium (NBT) dye. NBT reacts with "O^2 produced by the NADH/PMS system giving blue formazan which can be monitored at 560 nm.

Assay of catalase activity in serum.

Catalase activity was determined by the method of Chance and Mackely (1995). The rate of decomposition of H2O2 by catalase is measured by spectrophotometer at 240 nm.

Assay of whole blood glutathione peroxidase activity.

This was carried out by commercial glutathione peroxidase kits (RANSEL Randox Laborations Ltd., Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT 29 4QY). It is method based on the oxidation of GSH by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm is measured.

Converting to units/gm hemoglobin was done by dividing the obtained value in units/dL by Hemoglobin concentration (g/dL).

Statistical analysis:

Values are given as means ± SD, median (range), or as the number of subjects and proportions. One-way ANOVA test and Independent samples Student-*T* test were used for group comparisons of normally distributed variables. P value was considered significant<.05.

RESULTS

The study included 30 neonates with ages from 0- 1 month. They were 17 (56.6%) males and 13 (43.4%) females. Healthy controls were of cross matched ages (ranged from 0- 1 month) and sex (males 6 (54.4%) and females 5 (45.45%).

The commonest isolated organisms from patients' blood cultures were *staphylococci* 20 (66.7%) with *Staphylococcus aureus* from 12 patients (40.0%) and *Staphylococcus epidermidis* from 8 patients (26.7%). Other isolated organisms were *Escherichia coli (E.coli)* 4 (13.3%) and *Streptococcus pneumonia*e 3 (10%). Polymicrobial infections due to both *Staphylococci species* and *E.coli* were found in (10%), table 1.

Table 1: Distribution of sepsis patients according to the type of isolated organism from the blood culture:

	n (%)
(I) Staphylococci	20 (66.7%)
a) Staphylococcus aureus (Staph. aureus)	12(40.0)
b) Staphylococcus epidermidis	8(26.7)
(II) Other isolated organisms	10(33.3%)
Escherichia coli	4(13.3)
Streptococcus pneumoniae	3(10.0)
Polymicrobial (mixed) infection *(E.coli+ staphylococci)*	3(10.0
Total	30 (100.0)

There was highly significant decrease in erythrocyte superoxide dismutase (RBC SOD) (P<0.001) and whole blood glutathione peroxidase (GPx) (P=0.01) at admission and after 48 hours (P<0.001, for each) in patients compared with controls.

There was a significant decrease of red blood cells (RBCS) SOD (P=0.004) and blood GPx (P<0.001) after 48 hours from antibiotics intake compared with its level at admission (Table 2). The out come of sepsis was fatal for 17 patients (data not shown).

Table 2: Comparative study of superoxide dismutase, serum catalase and glutathione peroxidase (GPx) of patients compared with controls at admission and at 48 hours after admission:

Parameter	SOD (% inhibition)	Catalase (KU/mg protein)	GPx (U/g Hb)
(I) Control			
Mean + SD	50.00 + 4.21	1.12+0.23	55.82+12.14
n	(11)	(11)	(11)
(II) Patients			
a) at admission			
Mean + SD	18.47+8.34	2.59+1.18	39.21+18.22
n	(30)	(30)	(24)
P1	<0.001	<0.001	0.01
b) 48 hours			
Mean + SD	14.90+7.75	2.60+1.80	26.50+13.44
n	(30)	(30)	(26)
P2	<.001	.001	<.001
P3	.004	.408	<.001

P1: Patients enzyme at admission VS control.
P2: Patients enzyme 48 hours after admission VS control
P3: Patients enzyme at admission VS patients enzyme 48 hours after admission.

There was highly significant increase in serum catalase level in patients at admission (P<0.001) and after 48 hours compared with controls, with insignificant change in its level after 48 hours when compared with its level at admission (Table 3).

Interestingly, there was significant decrease of SOD (P1=.0001), GPx (P1=.049) at start of sepsis associated with *Staphylococcus aureus* infection. However, for *Staphylococcus epidermidis* and polymicrobial infections there

was only significant decrease in SOD (P2=.0001) and GPx had non significant decrease (P2=0.597, P3=0.539 respectively).

After 48 hours, both SOD and GPx had significant decrease with all types of bacteria isolated with significant decrease of GPx in *Staphylococcus epidermidis* and more decrease in polymicrobial infections(P4=.0.001). Catalase had significantly higher levels than control regardless the type of bacteria isolated at start and after 48 hours.

DISCUSSION

In the present study there was significant decrease in sepsis patients when compared with controls both at admission (P1=.01) and after 48 hours (P<.001).

Table 3: Kinetic changes of SOD. GPx and catalase in relation to the isolated bacteria.

Parameter	At admission			48 hrs after admission		
	R.B.C. SOD (%inhibition)	Serum catalase (KU/mg protein)	Whole blood GPx (U/g Hb)	R.B.C. SOD (%inhibitio n)	Serum catalase (KU/mg protein)	GPx (U/g H)
Control						
Mean +SD	50.00 + 4.21	1.12 + 0.23	55.82+12.14	- -	- -	- -
n	(11)	(11)	(11)			
Staph.aureus Mean + SD	19.76 + 8.47	2.82 + 1.22	34.44+17.56	17.47 + 7.99	2.97 + 1.97	29.00+16.3
n	(12)	(12)	(9)	(12)	(12)	(11)
P1	.0001	.0001	.049	.000	0006	.001
(III) Staph.epid Mean + SD	18.58 + 6.58	2.38 + 0.81	42.00+22.18	12.47 + 7.40	2.65 + 2.33	28.43+14.4
n	(8)	(8)	(7)	(8)	(8)	(7)
P2	.0001	.0001	0.597	.0001	.043	.001
(IV) Other organisms Mean + SD	16.83 + 9.87	2.47 + 1.42	42.13+16.43	13.17 + 7.57	2.12 + 1.01	21.39+6.43
n	(10)	(10)	(8)	(10)	(10)	(8)
P3	.0001	.006	.539	.0001	.004	< .001
P4	.727	.683	.054	.325	.559	< .001

P1: Patients with Staph aureus versus controls
P2: Patients with staph .epidermidis versus controls
P3: Patients with other organisms versus control
P4: Intergroup significance.

This result agrees with the result obtained by Goode and colleagues (1995) who reported decreased plasma glutathione peroxidase activity in sepsis patients.

Also, our result agrees with those of Angstwurm and colleagues (1999). On the other hand this result disagree with the results obtained by Batra and colleagues (2000) and Kapoor *et al.,* (2006) who reported a significant elevation of serum glutathione peroxidase activity in neonatal sepsis and attributed this elevation as a response to higher free radical production.

Another member of scavenger's pathway is catalase enzyme which is a protein enzyme present in most aerobic cells being especially concentrated in liver and erythrocytes. Although the activity of the enzyme does vary between different locations, the catalase enzyme can detoxify hydrogen peroxide by catalyzing the breakdown of hydrogen peroxide H2O2 according to the reaction (Batra *et al.,* 2000).

For serum catalase results, we found that serum catalase level was significantly increased in sepsis patients at admission and still significantly elevated at 48 hrs after admission when compared to controls (P1<.001).

This result agrees with the result obtained by Mikhalchik and colleagues (Mikhalchik, *et al.,* 2003). They attributed this increase to the effect of cytokines on radical production by phagocytes which when analyzed revealed a redistribution of the extra cellular and intracellular fractions of free radicals rather than a general increase of the oxygen active metabolite production. As expected, the increment in the number of intracellular radicals improved significantly the process of phagocytosis. So, the concentration of catalase increases extracellularly to prevent intracellular consumption of free radicals. Also, our results agree with the result obtained by Kharb and colleagues (2000) who reported that plasma and erythrocyte catalase activities were higher in septic shock patients when compared to controls.

The non significant change in catalase level between start and after 48 hours could be explained on the base that maximum redistribution of this enzyme occurs at start of infection.

Superoxide dismutase (SOD) is an enzyme system that appears to be specifically evolved to deal with hydrogen peroxide as a substrate and provides the second layer of defense after GPx and catalase against free radical injury. This enzyme catalyses the dismutation of superoxide ("O^2) to hydrogen peroxide (H2O2) (Batra *et al.,* 2000).

In our patients, erythrocyte superoxide dismutase (SOD) was highly significantly decreased in sepsis patients compared with controls at admission(P1<.001) and still significantly decreased after 48 hours(P2<.001). This result agree with the results obtained by Bela and colleagues, (2001) who reported that critically ill patients (including sepsis patients) have significantly low levels of SOD at the time of admission according to the severity of the prevalent clinical situation. Also, this

result agree with the results obtained by Leach and colleagues, (1998) that in sepsis there is an enhanced formation of reactive oxygen species in conjugation with inadequate defenses against such reactive oxygen species, due to endotoxaemia which result in a rapid, but transient, decline in the expression of both mRNA and protein of copper-zinc superoxide dismutas (Cu-Zn SOD).

On the other hand our result disagree with the result obtained by Batra and colleagues (2000) and Seema et al. (1999) who reported that there is significant increase in serum superoxide dismutase activity in neonatal sepsis, and they explained this increase as a response to higher free radical production. SOD, catalse, and GPx can be considered as complementary enzyme systems that can combine to limit oxidant stress on the cell from toxic oxygen intermediates. They also protect each other from oxidant inactivation. H2O2 can inactivate SOD and "O^2 can inhibit catalase and peroxidase function. Hence, SOD would be expected to prevent catalase and peroxidase inactivation, whereas these latter enzymes would protect SOD (Thang *et al.,* 2001).

In our study, it seems that there were much production of H2O2 which inactivate SOD, this resulted in increase "O^2 with inhibition of GPX and the only remaining active enzyme was the catalase due to its redistribution between intracellular and extracellular compartments. However, the role of catalase as scavenger enzyme is less important than GPX. This means that there was increase in free radicals with consumption of scavengers' enzymes which could be implicated in the pathogenesis of septicemia. These changes had occurred early during septicemia and could be used as early markers of septicemia.

Important finding of the present study that antibiotics therapy did not improve the decline in the levels of scavengers' enzymes and more over there was grave out comes of sepsis. So, the use of antioxiants supplements may be used in such conditions as adjuvant therapy to antibiotics to improve the out comes of sepsis.

In the study of blood culture results, the commonest isolated organism was *Staphylococcus* species. This result agrees with the result obtained by Wray *et al.*, (2001) and Huang *et al.,* (2003) who reported that the incidence of sepsis and septic shock due to Gram-positive organisms has increased dramatically over the last two decades.

The kinetics of scavenger enzymes seem to differ according to the type of the isolated bacteria. There was significant decrease of SOD (P1=.0001) and GPx (P1=.049) at start of septicemia in infection with *Staph. aureus*. After 48 hours, both SOD and GPx were significantly decreased with all types of isolated bacteria with significant decrease of GPX in *staph. epidermidis* and more decrease in polymicrobial infections.

The seriousness of staplylococcal infection is due to the increased production of free radicals which doesn't improve the efficacy of intracellular digestion of *staphylococci* (Kharb *et al.,* 2000). Moreover; it seems that the increase of free

radicals leads to rapid inactivation of the main scavenger enzymes which might aid to the increase in the pathogenesis of staphylococcal septicemia.

In conclusion, our patients had sepsis mostly due to *staphylococcus* species which resulted in oxidant stress with early depletion of the activity of scavengers enzymes superoxide dismutase and glutathione peroxidase. Also, these changes could be used as early predictors of septicemia. It seems that *staph. aureus* infection had the most pronounced effect of the depletion of these enzymes. So, these results suggest that those children were susceptible to high oxidative stress which may play a role in the pathogenesis of septicemia, so the utility of supplementation of antioxidant enzymes in neonates with septicemia needs further evaluation.

REFERENCES

Angstwurm MW; J. Scho -Hdorf., J. Schopohl 1999. selenium replacement in patients with severe systemic inflammatory response syndrome improves clinical outcome. Crit care Med; **27**(9): 1807–1813.

Batra S, Kumar R , Kapoor AK, Ray G. 2000. Alterations in antioxidant status during neonatal sepsis. Ann Trop Paediatr, **20:** 27–33.339

Bela P, Bahl R. ; Sane AS ; Sawant PH ; Shah V.R. ; Mishra VV and Trivedi HL 2001. oxidative stress status: possible guide line for clinical management of critically ill patients. Panmierva Medn, **43**(1): 27–31.350

Chance B. and Mackley A. 1995. Assays of catalases and peroxides. Methods Enzymol., **2:** 764– 75. 331

Dellinger R.P, Opal S M, Rotrosen D 1997. From bench to the bedside: The future of sepsis research.Chest.744-53.313

Friedman J.S.; Rebel V.I. ; Derby B. and Burrako F.F. 2001. Absence of mitochondrial superoxide ismutase results in a hemolytic anaemia responsive to therapy with a catalytic antioxidant. J ExpMed.. **193:**925-34.

Goode H F.; Cowley HC. ; Walker B E ; Howdle PD and Nebster NR 1995 Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. Crit Care Med., **23**(4): 646-651.334

Gutteridge J.M.C and Mitchell J. (1999): Redox imbalancein the critically ill. Br Med Bull ; 55: 49 – 75. 324

(U/g H*Huang S.Y.; Tang R.B. ; Chen S.J. and Chung R.L.* 2003. coagulase- negative staphylo coccal bacteremia in critically ill children, risk factors and antimicrobial susceptibility. J Microbiol Immunol Infect; **36**(1): 51–55.

(U/g HKapoor K, Basu S, Das BK, Bhatia BDLipid peroxidation and antioxidants in neonatal septicemia. : J Trop Pediatr. 2006; **52**(5):372-5. 322

(U/g HKharb S.; Singh V.; Ghalaut P.S.; *et al.,* 2000. Role of oxygen free radicals in shock. J Assoc physicians India; **48**(10): 956–7. 347

(U/g HLeach M.; Frank S.; Olbrich A.; *et al.,* (1998): Decline in the expression of copper\zinc superoxide dismutase in the kidney of rats with endotoxic shoch: effects of superoxide anion radical scavenger, tempol on organ injury. Br J pharmacol, **125**(4): 817–825. 354

(U/g HMcDonald M.C. ; d'Emmanuele divilla Bianca R, Wayman N.S. , Pinto A. , Sharpo M.A. , Cuzzocrea S. , Chatterjee P.K. , Tniemerma NNC (2003) A Superoxide dismutase mimetic with catalase activity (EUK-8) reduces the organ injury in endotoxic shock; **466**(1-2): 181–189. 320

Michel M and Gutmann (1997)Methicillin resistant staphylococcus aureus and vancomycin resistant enterococci:Therapeutic realities and possibilities. Lancet. **349:**1901-1906.

Mikhalchik E.V. ; Kharaeva Z.F. ; Kovalchuk L.V. ; *et al.,* 2003. Effect of cytokines on the level of free radicals in the blood of patients with systemic and and local staphylococcus infection. *Russ J. Immunol*; **7**(3):251–8.345

Nishikimi, M. ; Rao N. A., Yagi K. 1972. Occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen . Biochemo Biophys. Res. Commun., **46:**849-329

Seema; Kumar R., Mandal R.N., Tandon A., Randhaua V.S., Mehta G., Batra S. and Kapoor A.K.C. 1999. Serum TNF alpha and free radical scavengers in neonatal sepsis. Indian J. Pediatr., **66**(4): 511-516 357

Thang P.T.; Patrick S.; Teikl S *et al.,* (2001): Antioxidant effects of the extracts from leaves of chromolaelna odorata on human dermal fibroblasts and epidermal keratinocytes against hydrogen. Peroxide and hypoxanthine oxidase induced damage. Burn. **27:**319-327. 361

Tollner U (1982): Early diagnosis of septicaemia in the newborn:clinical studies and sepsis score.Eur.J.Pediatr.331-7. 326

Wray G.M., Faster S.J., Hinds C.J., *et al*.,(2001) : A cell wall component from pathogenic and mon-pathogenic Gram-positive bacteria (peptidoglycan) Synergises with endotoxin to cause the release of TNF- alpha, nitric oxide production, shock and multiple organ injury\ dysfunction in the rat. Shoch; (2):135–142.