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Original Article

Oxidant and antioxidant status in neonatal proven and clinical sepsis according to selenium status

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Abstract ***Background:*** Selenium is a trace element required for the functioning of the immune system. Neonatal sepsis is a serious condition leading to morbidity and mortality in neonates worldwide. The purpose of this study was to measure selenium and plasma selenoprotein P (SePP), selenoenzyme activity, and alterations in oxidant/antioxidant status with immune biomarkers in neonates with clinical (n = 27) and proven neonatal sepsis (n = 25).

Methods: Erythrocyte selenium and SePP; plasma lipid peroxidation (LP), protein oxidation and total antioxidant capacity and erythrocyte total glutathione (GSH) concentration; erythrocyte glutathione peroxidase (GPx), thioredoxin reductase (TrxR), catalase (CAT) and total superoxide dismutase (SOD) activity were measured spectrophotometrically/spectrofluorometrically. Plasma interleukin 2 and 6 were also measured.

Results: Erythrocyte selenium and SePP were markedly lower both in the clinical and proven sepsis groups versus control. Erythrocyte GPx activity was higher only in the clinical sepsis group. TrxR activity was markedly lower in proven sepsis. SOD activity and GSH were markedly higher both in clinical sepsis and in proven sepsis. CAT activity was significantly higher both in clinical sepsis and in proven sepsis. LP and protein oxidation were significantly higher in both of the sepsis groups.

Conclusions: Both selenium-dependent and selenium-independent blood redox systems were altered in sepsis, suggesting that sepsis causes an imbalance between cellular antioxidant and oxidant states.

Key words antioxidant enzyme, clinical sepsis, oxidative stress, proven sepsis, selenium, selenoenzyme.

Neonatal sepsis is a serious condition leading to morbidity and mortality in neonates worldwide, particularly in developing countries.[1,2] The gold standard for the diagnosis of neonatal sepsis is blood culture. If growth is observed, this condition is termed "proven sepsis".[3] Blood culture results, however, which are needed to provide accurate diagnosis, take at least 48–72 h and occasionally produce false negatives. Early and accurate diagnosis of newborn sepsis is necessary to decrease sepsis mortality, morbidity and to avoid unnecessary use of antibiotics. Prolonged premature rupture of membranes >24 h, Apgar score <6 at 5 min, very low birthweight, prematurity (gestational age <37 weeks) and mechanical ventilation are independent risk factors for sepsis.[1] Neonates are highly susceptible to infectious diseases because of their immature immune systems, particularly impaired humoral defenses, and poorly developed skin barrier.[4] Also, poor maternal nutrition resulting in low birthweight is classified as another risk factor.[5] Hence, in several infectious diseases, the role of nutrition has long been linked to alterations in immune response.[6] Nutritional deficiency, particularly deficiency of trace elements such as selenium, can lead to susceptibility to a wide variety of infections.[7]

Selenium is a crucial essential dietary element required for the regulation of cellular redox homeostasis and the functioning of neutrophils, macrophages and natural killer cells.[8] Moreover, selenium deficiency is associated with an increase in several infectious conditions.[8]

Selenoenzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) contain selenocysteine at their active centers. GPx degrade a variety of peroxides. GPx1 is found in many tissues and erythrocytes, while GPx3 is expressed by several types of epithelia and is secreted into plasma by the kidney.[9] The thioredoxin system is an important defense system against oxidative stress and is involved in many biological processes, such as DNA metabolism and repair, and regulation of several transcription factors and signal transduction.[10,11]

Selenoprotein P (SePP), the main plasma selenoprotein, may have high antioxidant potential, and binds to endothelium. It is postulated that in septic shock, and similar syndromes such as systemic inflammatory response syndrome, SePP binds massively to endothelium, causing a drop in SePP plasma concentration.[12] SePP,

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rather than GPx, is regarded as a potential marker of septic shock and related syndromes.[12]

Other components of cellular antioxidant defense, namely superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH), are also required for the reduction of cellular reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot-}$) and H_2O_2. Lipids constitute an important class of biomolecules among the molecular targets of ROS.[13] Lipid peroxidation (LP) can greatly alter the physicochemical properties of membrane lipid bilayers, resulting in severe cellular dysfunction. It is well established that end products of LP, such as malondialdehyde (MDA), can lead to protein oxidation.[14] In contrast, C-reactive protein (CRP), procalcitonin (PCT), interleukin 2 (IL-2) and interleukin 6 (IL-6) are the most widely studied biomarkers and are considered to be the most promising for diagnosing neonatal infections.[15]

To our knowledge, there has been no study on selenium, selenoenzymes, SePP, and alterations in oxidant/antioxidant status with regard to immune biomarkers in both neonatal clinical and proven neonatal sepsis. The aim of this study was therefore to assess the role of oxidant/antioxidant status and selenium in septic neonates with the biomarkers of sepsis.

Methods

Chemicals and reagents

All chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA).

Kits

Colorimetric assay kits for TrxR and GSH were from Sigma-Aldrich. Antioxidant assay kit and carbonyl kit were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). SePP kit was from Cusabio (Hubei, China). IL-2 and IL-6 kits were from Abcam (Cambridge, MA, USA).

Equipment

All high-performance liquid chromatography (HPLC) equipment was from Agilent (Santa Clara, CA, USA).

Study groups

The study was designed as a case–control study and focused on oxidant and antioxidant status in neonatal sepsis in relation to selenium status. The study group consisted of 52 patients hospitalized in the Neonatal Intensive Care Unit of Hacettepe University İhsan Doğramacı Children's Hospital with a diagnosis of neonatal sepsis during 2012–2013. Infants with any of the following conditions were excluded from the study: maternal collagen tissue disease, HIV, syphilis, Toxoplasmosis, Other [T. pallidum, Varicella-zoster virus (VZV), Parvovirus B19], Rubellavirus, Cytomegalovirus (CMV), and Herpes Simplex Virus (HSV) syndrome, perinatal hypoxia/asphyxia, hypoxic ischemic encephalopathy (Apgar score <6 at 5 min, cord pH < 7.00, base excess > 10 mEq/L), chromosomal anomalies, major congenital malformations, multiorgan failure due to reasons other than sepsis, major rhythm disturbance, inherited metabolic disease, immune/non-immune hydrops fetalis, twin-to-twin transfusion syndrome, hematologic disease, malign tumor and surgery before diagnosis of sepsis.

Sepsis is diagnosed on clinical (hypothermia, hyperthermia, respiratory distress and shock) and laboratory (positive CRP, PCT, immature–total leukocyte ratio (IM/T), blood culture) findings. Twenty-five patients with positive blood culture comprised the proven sepsis group, while 27 neonates with negative culture were recruited to the clinical sepsis group. The control group consisted of 35 healthy neonates matched for gestational age. Written informed consent was obtained from the parents of the children and the study was approved by Hacettepe University Human Ethics Committee.

Hemoglobin (Hb), hematocrit, platelet count, and total leukocyte count were measured using arbitrary units (au) by automated analyzer (Beckman-Coulter, Pasadena, CA, USA) according to the manufacturer's instructions. Absolute leukocyte count and IM/T were calculated by examination of peripheral blood smear. Serum CRP and PCT were measured daily on nephelometry (Beckman-Coulter) and fluorometric immunoassay (Kryptor; Brahms, Hennigsdorf, Germany), respectively.

Sample preparation

Venous blood samples were taken in heparinized tubes and centrifuged at $800 \times g$ for 15 min. Plasma was separated; erythrocytes were washed with 0.9% NaCl and centrifuged at $800 \times g$ for 10 min. This procedure was repeated three times and erythrocyte packages were obtained. Both plasma and erythrocyte samples were aliquoted and kept at –80 °C until analysis.

Plasma IL-2 and -6

Plasma IL-2 and IL-6 were measured using commercial kits. The standards or samples were added on biotinylated monoclonal antibody-coated plates specific for IL-2 or IL-6 and incubated. Later, streptavidin-horse radish peroxidase (HRP) was added, incubated and washed. *3,3*',*5,5*'-Tetramethylbenzidine (TMB) substrate solution was then added. The intensity of these colored products was measured at 450 nm.

Selenium and oxidant/antioxidant parameters

All spectrophotometry was carried out with a Schimadzu UV-1601 spectrophotometer (Kyoto, Japan).

The activity of erythrocyte GPx1 was measured in a coupled reaction with glutathione reductase (GR).[16] The assay is based on the instant and continuous reduction of GSSG, and was monitored spectrophotometrically at 340 nm. One unit of enzyme was defined as the amount of GPx1 that transformed 1 µmol of NADPH to NADP per min at 37 °C.

Erythrocyte TrxR activity was determined using the Thioredoxin Reductase Assay kit, with 5,5'-dithiobis-(2-nitrobenzoic acid (DTNB) as the substrate at 412 nm.[17] One unit of TrxR activity was defined as the amount of enzyme that caused an increase in absorbance of 1.0/min/mL at pH 7.0 at 25 °C.

A single-beam atomic absorption spectrometer (Perkin Elmer AAS Spectrometer 700, Waltham, MA, USA) with Zeeman background correction equipped with an Fs-go plus furnace autosampler was used in the measurement of plasma Se. Samples

and standard solutions were wet-injected into the graphite furnace. Three standard additions and peak height measurements were used for quantification.[18]

Plasma SePP was measured with a quantitative sandwich enzyme immunoassay kit. The intensity of the color was measured at 450 nm.

Erythrocyte CAT activity was determined according to Aebi (1974).[19] The enzymatic decomposition of H_2O_2 was followed directly at 240 nm. One unit of CAT activity was defined as the amount of enzyme required to decompose 1 μmol H_2O_2 in 1 min.

The total SOD activity in erythrocytes was determined by monitoring the auto-oxidation of pyrogallol at 420 nm according to Marklund and Marklund (1974) with some modifications.[20] One unit of total SOD activity was defined as the amount of enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%.

Erythrocyte GSH content was assessed with the Glutathione Assay Kit using DTNB as a substrate at 412 nm.[21] Quantification was achieved by reference to a standard curve of known GSH concentration, and results are expressed in nmol/mg Hb.

As an indicator of LP, MDA was measured on HPLC after reaction with thiobarbituric acid. Quantification was carried out by reference to a standard curve of known MDA concentration, and MDA is expressed as μmol/L.[22]

The plasma carbonyl groups as the biomarker of protein oxidation were measured with a commercial kit that relies on the 1:1 binding of fluorophore to protein carbonyls. Fluorescence was measured (excitation wavelength, 480 nm; emission wavelength, 535 nm), and the results are expressed as nmol/mL.

The assay relied on the ability of antioxidants in the sample to inhibit the oxidation of 2,2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) to $ABTS^+$ by metmyoglobin at 405 nm. The capacity of the antioxidants in the sample to prevent $ABTS^+$ oxidation was quantified as Trolox equivalent antioxidant capacity (mmol/L).

Statistical analysis

Results are expressed as mean ± SD. Between-group differences were analyzed using Student's *t*-test for normally distributed data or Kruskal–Wallis test for non-normally distributed data, with SPSS version 17.0 (SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant. Correlations were analyzed using Pearson or Spearman correlation coefficients.

Results

Subject characteristics

Gestational age, gender, birthweight, delivery type and other characteristics are summarized in Table 1.

C-RP, IM/T, PCT and Hb

C-reactive protein and PCT were statistically significantly high in the clinical and proven sepsis groups, as expected (Table 1). In the control group, only one subject had IM/T > 0.2; while in the clinical and proven sepsis group, 17 and 23 patients had IM/T > 0.2, respectively. Mean Hb was markedly lower in both the clinical and proven sepsis groups compared with the control (data not shown).

Plasma IL-2 and -6

Plasma IL-2 and IL-6 are given in Table 1. As expected, IL-2 was markedly higher both in the clinical (42%) and proven sepsis groups (72%), and IL-6 was higher in the clinical sepsis group (23%, $P = 0.000$) and proven sepsis group (51%, $P = 0.000$) compared with the control.

Oxidant/antioxidant parameters

Erythrocyte selenoenzyme (GPx and TrxR) activity, erythrocyte selenium and plasma SePP are given in Figure 1. GPx activity was higher only in the clinical sepsis group compared with the control (32%, $P = 0.004$). TrxR activity was markedly lower in the proven sepsis group (25%) compared with the control. Erythrocyte selenium was lower both in the clinical (15%, $P = 0.049$) and the proven sepsis groups (16%, $P = 0.040$) compared with the control. SePP was significantly lower in both the clinical sepsis (61%, $P = 0.000$) and in the proven sepsis groups (71%, $P = 0.000$).

Antioxidant enzyme activity, total GSH, MDA, protein carbonyl and total antioxidant capacity are shown in Figure 2. Total SOD activity was markedly higher both in the clinical sepsis group (30%, $P = 0.02$) and proven sepsis group (45%, $P = 0.04$) compared with the control group. CAT activity was higher both in the clinical sepsis group (27%, $P = 0.002$) and the proven sepsis group (29%, $P = 0{,}004$) versus the control. Total GSH was only markedly lower in the proven sepsis group (29%, $P = 0.006$). LP was significantly higher in both the clinical sepsis group (17%, $P = 0.03$) and the proven sepsis group (22%, $P = 0.04$) versus the control. Protein oxidation expressed as protein carbonyl was higher in both the clinical sepsis group (34%, $P = 0.002$) and proven sepsis group (37%, $P = 0.0000$) compared with the control. Total antioxidant capacity was not different compared with the control in any of the study groups.

Correlations

Correlations between immune parameters were analyzed using Pearson or Spearman correlation coefficients. In both the proven and clinical sepsis groups, CRP was strongly correlated with both IL-2 and IL-6 ($P < 0.05$, both). In the clinical sepsis group, CRP was positively correlated with PCT.

Selenium and SePP were positively correlated in both the proven sepsis and clinical sepsis patients (r = 0.321 and r = 0.302, respectively; $P = 0.002$). We also observed a positive correlation between TrxR activity and SePP in both the proven sepsis and clinical sepsis groups (r = 0.356 and r = 0.405, respectively; $P = 0.001$; data not shown).

Discussion

Neonatal sepsis is classified as one of the leading causes of mortality.[3] Sepsis in neonates is generally categorized as early or late onset. Early-onset neonatal sepsis occurs within the first 72 h of life, while the late-onset neonatal sepsis occurs after 72 h.[23] In the present study, all of the patients had late-onset sepsis.

Table 1 Subject characteristics and inflammation parameters

Groups	n	Age (days)	Maternal age (years)	Gestation (weeks)	Birthweight (g)	Birth type (normal/cesarean)	Hospitalization (days)
Control (17 girls, 18 boys)	35	7 ± 5.80†	28.38 ± 5.19†	34.85 ± 4.23†	2556.9 ± 752†	7/28	5.22 ± 4.69†
Mean ± SD							
Median (range)		6 (1–22)	28 (20–40)	36.5 (32.10–41)	2550 (1100–4270)		3 (0–21)
Clinical sepsis (10 girls, 17 boys)	27	15.13 ± 11.84‡	28.75 ± 6.05†	34.48 ± 4.56†	2202.9 ± 1021.3†	11/16	23.17 ± 20.47‡
Mean ± SD							
Median (range)		14 (1–45)	29 (19–44)	36 (27.30–41.50)	2510 (810–4050)		13 (0–76)
Proven sepsis (12 girls, 13 boys)	25	13.13 ± 9.31‡	28.77 ± 5.85†	36.27 ± 2.59†	2425.3 ± 960.1†	7/18	25.54 ± 20.56‡
Mean ± SD							
Median (range)		12 (2–39)	28 (18–42)	36.5 (32.10–41)	2530 (778–4270)		20 (6–92)

†, ‡, §Significantly different from each other ($P < 0.05$). CRP, C-reactive protein; IL, interleukin; PCT, procalcitonin.

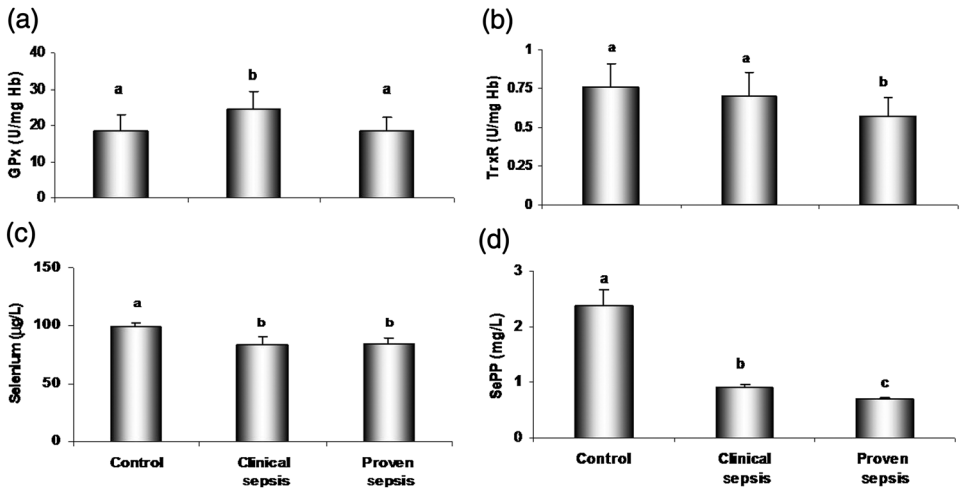

Fig. 1 Erythrocyte selenoenzyme (a, glutathione peroxidase [GPx]; b, thioredoxin reductase [TrxR]), (c) erythrocyte selenium and (d) plasma selenoprotein P (SePP) in neonates. [a,b,c]Significantly different from each other ($P < 0.05$).

White blood cell count, IM/T, absolute leukocyte count and acute phase reactants such as CRP, PCT, and IL-6 are now the most frequently used markers for the diagnosis of newborn sepsis.[24] In the present study, both CRP and PCT in both of the study groups were found to be significantly higher compared with the control. IM/T ratio in both clinical and proven sepsis patients was significantly higher compared with the control, indicating the importance of this biomarker in septic neonates.

Certain bacterial pathogens are able to alter oxidative immune response and the balance between oxidants and antioxidants in the host.[25] The production of ROS and reactive nitrogen species (RNS) is an important host defense mechanism in response to infection by bacterial pathogens. Intracellular pathogens, however, have evolved numerous defense strategies to protect themselves against the damaging effects of ROS and/or RNS.[26] There has been ongoing interest in the role of systemic oxidative stress in sepsis in the last decades.[27] ROS can cause direct cellular injury by oxidative injury and/or can induce LP. This may result in cellular membrane damage. Moreover, ROS can directly interfere with the regulation of pro-inflammatory cytokine genes, and hence in the ongoing activation of the inflammatory response to infection.[28]

The consequences of persistent bacterial infection potentially include increased morbidity and mortality from the infection itself, as well as an increased risk of dissemination of disease. Many studies have demonstrated increased oxidant activity and/or decreased antioxidant defenses in septic patients.[29,30] As a key component of endogenous antioxidant defense systems, several studies have determined that plasma selenium concentration was markedly reduced in patients with septic shock and in critically ill patients.[31,32] Dietary selenium is suggested to be essential for optimum immune response, and selenium deficiency may affect both cell-mediated and humoral components of immune response.[33] Some studies have shown that selenium intake and status can affect the progression of viral infections, and suggest an association between host nutritional Se status and viral infection.[34]

Several studies and comprehensive reviews have noted that interpretation of plasma selenium concentration can be difficult for several reasons. First, a normal acute phase response can be the cause of the decrease in plasma selenium concentration, and should be interpreted in combination with a marker of systemic inflammation, for example, CRP.[24] Second, plasma concentration represents the sum of different selenium-containing compounds, and although there are data on the

Table 1 (Continued)

Groups	Sepsis diagnosis (days)	CRP (mg/L)	PCT (μg/L)	Platelet count (cell/ml)	IL-2 (pg/mL)	IL-6 (pg/mL)
Control (17 girls, 18 boys) Mean ± SD	–	0.15 ± 0.06†	0.80 ± 0.24†	249 739 ± 41 623†	155.33 ± 26.63†	60.09 ± 7.79†
Median (range)		0.15 (0.10–027)	0.56 (0.10–2.25)	245 000 (142 000–466 000)	134.33 (45–251.50)	59.74 (2.12–129.90)
Clinical sepsis (10 girls, 17 boys) Mean ± SD	13.89 ± 10.46†	1.51 ± 0.26‡	10.84 ± 1.83‡	278 485 ± 47 072†	221.06 ± 16.37‡	73.99 ± 9.67†‡
Median (range)	12 (1–45)	0.40 (0.10–10.50)	0.90 (0.10–99)	254 000 (32 000–668 000)	212.33 (13.16–385.33)	54.41 (21.91–167.28)
Proven sepsis (12 girls, 13 boys) Mean ± SD	13.86 ± 11.62†	4.15 ± 0.72§	10.25 ± 1.78‡	259310 ± 42630†	267.25 ± 55.19‡	90.80 ± 21.87‡
Median (range)	12 (1–43)	1 (0.10–24.50)	0.97 (0.13–174)	249 000 (502–618 000)	221.41 (120.33–361.66)	44.02 (19.40–301.33)

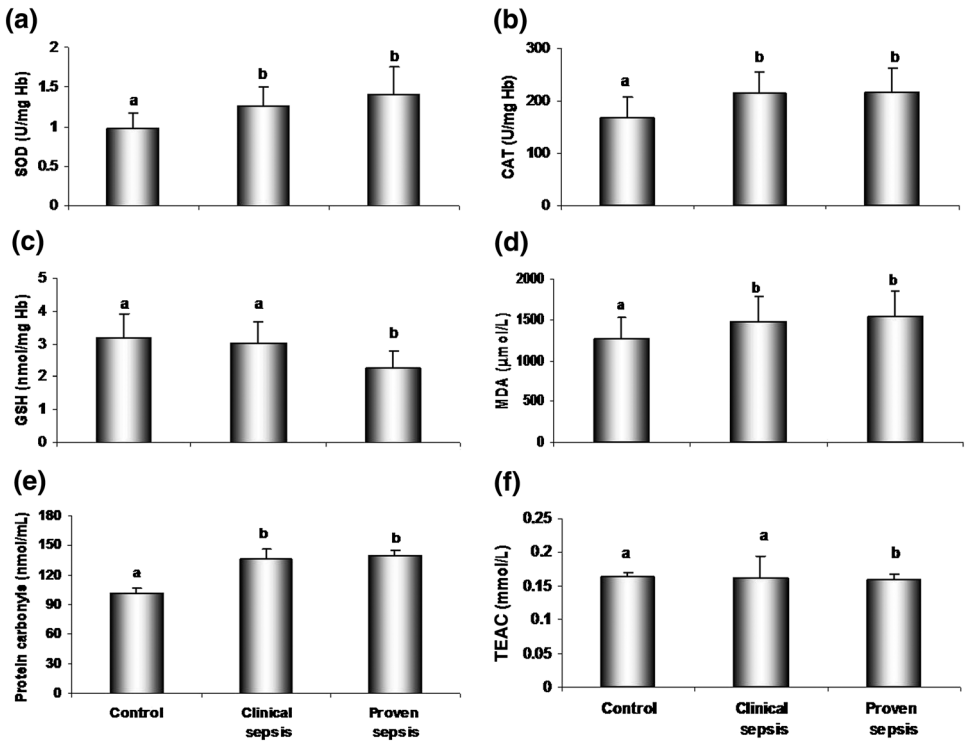

Fig. 2 (a) Erythrocyte superoxide dismutase (SOD), (b) erythrocyte catalase (CAT), (c) erythrocyte total glutathione (GSH), (d) plasma malondialdehyde (MDA), (e) plasma protein carbonyl and (f) total Trolox equivalent antioxidant capacity (TEAC). MDA reflects lipid peroxidation; protein carbonyl reflects protein oxidation. Data given as mean ± SEM. [a,b]Significantly different from each other ($P < 0.05$).

relative proportions of these compounds in healthy individuals, absolute values are not known in critically ill patients. Third, albumin concentration diminishes in critically ill patients, possibly due to increased endothelial permeability, leading to loss of proteins including SePP. This phenomenon may contribute to the decreased selenium concentration seen in these patients.[12] Fourth, plasma selenium concentration may not accurately reflect tissue selenium content or selenoenzyme activity. Finally, it has been observed that, worldwide, selenium status is not in the optimal range (70–150 μg/L), but rather a marginal status has been noted, compared with the USA where intake is higher.[35,36] Therefore, rather than a real deficient state, except in those with malnutrition, the relative dietary insufficiency observed most probably does not contribute to the low selenium level seen in critically ill patients. As a result, we measured erythrocyte selenium concentration because this reflects long-term selenium intake, given that the erythrocyte lifespan is approximately 3 months.[37] In the present study, given that we studied neonatal selenium, measurement of neonate erythrocyte selenium might reflect the transition of selenium from mother to child, thereby also enabling us to clarify the role of selenium in these neonates. Erythrocyte selenium was markedly lower in both the proven (15%) and clinical sepsis patients (16%) compared with the controls. In patients with septic shock, Forceville *et al.* observed low plasma selenium concentration, which was correlated with the severity of illness.[31] Also, organ system failure rate and mortality rate were higher (approx. threefold) in patients with low plasma selenium concentration (selenium

≤0.70 mmol/l) at the time of admission than in patients with normal selenium level. More recently, Sakr *et al.* reported that 92% of consecutive surgical intensive care unit patients (n = 60) had lower plasma selenium concentration compared with healthy subjects.[32]

In the current study, plasma SePP was significantly lower in both of the study groups compared with the control. In healthy individuals, SePP is the major selenoprotein in plasma, accounting for 40–70% of the total plasma selenium, and GPx accounts for another 20–40%.[38] GPx activity is a crucial factor in the course of infectious disease. We did not observe any alteration in erythrocyte GPx activity in the proven sepsis group, but it was significantly higher in the clinical sepsis group versus the control. This might be an adaptive response toward an oxidative insult. TrxR activity, however, was markedly lower in the proven sepsis group compared with the control, suggesting that these two selenoenzymes are differently regulated in these two conditions.

We also observed increases in SOD and CAT activity in both the clinical sepsis and proven sepsis groups, with the increase in SOD activity being more pronounced in the proven sepsis group. This suggests that these increases might also be adaptive alterations, but it is difficult to postulate whether these changes reflect increases at a transcriptional or translational level.

Glutathione, the major thiol in most living cells, is the key antioxidant in animal tissues.[21] LP results in severe cellular dysfunction, and it is widely known that MDA and other end products of LP can lead to protein oxidation.[14] Erythrocyte total GSH was markedly lower in the proven sepsis group, while plasma LP and protein oxidation were significantly higher in both of the study groups. In contrast, total antioxidant capacity was found to be markedly lower only in the proven sepsis group. All these alterations indicate that there is an imbalance in oxidant/antioxidant status in the two sepsis groups.

Selenium supplementation seems to be beneficial in septic or critically ill patients, as shown in some interventional studies. A prospective randomized clinical trial of 24 critically ill patients investigated plasma selenium, MDA, GSH, IL-2R, IL-6 and IL-8 with and without parenteral selenium supplementation. Following 24 h of supplementation, selenium plasma normalized, and MDA decreased significantly beginning at day 3. Excessive stimulation of the immune system was not seen on the applied dosage.[39] A prospective, multi-center, placebo-controlled, randomized, double-blind study with an intention-to-treat analysis was performed in severe septic shock patients with documented infection. Sixty patients received selenium for 10 days (4000 μg on the first day, 1000 μg/day as Na selenite on the 9 following days) or matching placebo using continuous i.v. infusion. The primary endpoint was the time to vasopressor therapy withdrawal. The median time to vasopressor therapy withdrawal was 7 days in both groups, but mortality rate did not significantly differ between the groups at any time point. Selenium had no obvious toxicity but did not improve clinical outcome in the septic shock patients.[40] In a prospective randomized study by Zimmermann *et al.*, the effect of sodium selenite over a period of 28 days was investigated in 20 patients with systemic inflammatory response syndrome and multiple organ failure. At the end of the study, sodium selenite therapy was associated with a reduction in the mortality rate from 40% to 15%.[41]

The current study has some limitations: (i) the number of subjects in the study groups could have been increased; (ii) the expressions of selenoenzymes (GPx1 and TrxR) could have been measured in order to determine whether the effects of sepsis emerge in transcription, translation or after translation; and (iii) other LP parameters, such as F2-isoprostane, could have been measured.

Conclusions

Both selenium and SePP were low in the two sepsis groups, indicating the role of selenium and selenoproteins in neonatal sepsis. Also, in neonatal sepsis, oxidative stress is evidenced by alterations in antioxidant enzyme activities, decreases in the most important cellular thiol GSH and increases in LP and protein oxidation. It is difficult to specify whether a high oxidative state is the cause or consequence of sepsis, but neonatal sepsis can be suggested to be related to oxidative stress, thus triggering changes in cellular antioxidant/oxidant balance. This study therefore has provided evidence that selenium and SePP should be of particular concern, and that measurement of alterations in antioxidant enzymes and cellular oxidation state can indicate a diagnosis of sepsis in neonates. Randomized controlled studies are needed to determine whether selenium treatment can change the course of sepsis in neonates and premature infants.

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