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PEDIATRIC BRIEF REPORT

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Persistently low plasma thioredoxin is associated with meningococcal septic shock in children

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Abstract *Objective:* To compare plasma levels of thioredoxin (Trx), TNF-α and IL-1β in children during the acute phase of meningococcal septic shock (MSS) and in convalescence. *Design and setting:* Retrospective, observational study in the paediatric intensive care unit of a postgraduate teaching hospital. *Patients:* Thirty-five children requiring intensive care for meningococcal sepsis; paired convalescent samples from 30 survivors (median interval between samples 62 days); 25 healthy control children. *Measurements and results:* Plasma Trx levels were significantly lower in the children with MSS, both during the acute illness (5.5 ng/ml, IQR 1.4–11.4) and in convalescence (2.5 ng/ml, IQR 0.4–6.9) than controls (18.8 ng/ml, IQR 7.9–25.0). Levels of IL-1β and TNF-α were higher in patients with acute MSS (30.3 pg/ml, IQR 3.6–63.6, and 145.9 pg/ml, IQR 31.8–278.1 respectively) than controls (3.7 pg/ml, IQR 0–36.9, and 23.8 pg/ml, IQR 0–124.3, respectively). Levels fell in convalescence (3.7 pg/ml, IQR 0–25.5, 3.7 pg/ml, IQR 0–304.8, respectively). Plasma Trx was higher in non-survivors, albeit a small group ($n = 5$), than in survivors ($n = 30$). Trx, IL-1β, and TNF-α levels were not correlated with predicted mortality as assessed by the paediatric risk of mortality (PRISM) score. *Conclusions:* Children with MSS exhibit persistently low plasma levels of Trx during acute illness and in convalescence.

Keywords Meningococcal infection · Sepsis syndrome · Systemic inflammatory response syndrome · Thioredoxin · Oxidation-reduction · Cytokines

Introduction

Meningococcal septic shock (MSS) is a rapidly progressive devastating condition in which endotoxin released from the Gram-negative bacterium *Neisseria meningitidis* triggers an overwhelming systemic inflammatory response, activation of complement and coagulation pathways and circulatory collapse. Prompt administration of antibiotics, aggressive resuscitation and early transfer to a specialist centre are the mainstay of treatment [1]. A greater understanding of the pathology of MSS could help in the development of effective pharmacotherapy for MSS or, indeed, identifying those at-risk for a poor outcome. Our previous work suggests that during acute illness children with MSS also have compromised antioxidant protection; specifically, decreased protection against iron-catalysed oxidative damage which is correlated with disease severity [2]. Thioredoxin (Trx) is a 12-kDa ubiquitous thiol (-SH) protein that has potent anti-oxidant and cell activating properties that include modulation of inflammation [3]. Extracellular Trx is normally raised in a wide range of diseases associated with oxidative stress and inflammation such as viral infection, autoimmune conditions, heart disease and ischaemia-reperfusion injury. Trx is also related, although variably, to disease markers and severity/outcome [4]. The role of Trx in MSS has not been studied.

The aim of this study was therefore twofold: (a) to assess whether plasma Trx levels in children with MSS change during the acute and convalescence phase by comparison with TNF-α and IL-1β, and (b) to determine whether plasma level of Trx relate to severity of disease and outcome.

Materials and methods

Thirty five children with confirmed MSS admitted to the Paediatric Intensive Care Unit (PICU) of St Mary's Hospital London (from February 1998 to February 2003) were studied. The children were recruited consecutively. However, only those from whom adequate sample was collected were included in the study. Approval for collection and analysis of samples was obtained from the local research ethics committee. Informed written parental consent was obtained for all children recruited.

MSS was identified at presentation by fever, haemorrhagic rash and shock. Diagnosis was confirmed using blood cultures, latex agglutination testing of serum, a positive polymerase chain reaction for meningococcal DNA, or a rise in specific antibody in convalescent serum. Twenty-five healthy children were recruited consecutively either from ($n = 9$) or by ($n = 16$) the index family over the same 5-year period. This ensured that controls were from a similar geographic location and socioeconomic class and thus had a similar risk of exposure to meningococcus as the index case. Patients with MSS were significantly younger than controls [median 2.1 years, interquartile range (IQR) 0.9–4.3, vs. 6.8 years, IQR 2.3–9.4]; however, there was no relationship between plasma Trx and age either within any study group or when analysed together (data not shown). Samples from both study groups were collected under identical conditions. Illness severity was assessed using the Paediatric Risk of Mortality (PRISM) score for all children with MSS [5]. Patients with MSS were arbitrarily classified according to predicted mortality of less than (mild/moderate MSS) or greater than (severe MSS) 50%.

Blood was collected, spun down, separated and stored at -80°C within 12 h of admission to PICU and within 24 h of onset of illness. Trx levels were stable in stored samples and a limited number of freeze-thaw cycles had no effect on Trx levels [6]. Plasma Trx, TNF-α, and IL-1β were measured on samples taken within 12–24 h of onset of illness and during convalescence (median interval between samples 62 days; range between samples 39–463 days; IQR, 55–73 days) and from healthy control children. Trx, TNF-α and IL-1β were measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Redox Biosciences, Kyoto, Japan; and R&D Systems, Abingdon, UK). Trx concentrations were corrected for the effects of haemolysis by measuring free haemoglobin using a colorimetric kit (Sigma Diagnostics, Poole, UK) because erythrocytes have high Trx levels relative to the amount found in plasma [7]. Plasma Trx was then calculated as others and we have previously described [6, 8]. The standard curve for the Trx ELISA was from 7.8 to 126 ng/ml, and whilst all data points were on the linear part of the curve, some final concentrations were below 7.8 ng/ml because the contribution to Trx levels from haemolysed red cells was subtracted. Data are expressed throughout as median values and IQR and analysed using non-parametric analysis. Comparisons between groups were made using the two-tailed Mann–Whitney U-test. Levels of $p < 0.05$ were considered statistically significant.

Results

Plasma Trx was significantly lower in patients with acute MSS than in healthy children (5.5 ng/ml, IQR 1.4–11.4, vs. 18.8 ng/ml, IQR 7.9–25.0, $p < 0.01$; Fig. 1A). By contrast, plasma IL-1β was higher in patients with MSS than in controls (30.3 pg/ml, IQR 3.6–63.6, and 3.7 pg/ml, IQR 0–36.9, $p < 0.05$, respectively; Fig. 1B). Likewise, TNF-α levels were significantly higher in acute disease than in controls (145.9 pg/ml, IQR 31.8–278.1, and 23.8 pg/ml, 0–124.3, $p < 0.05$, respectively; Fig. 1C).

Comparisons were also made between the levels of each inflammatory marker in the acute vs. the convalescence phase. Thus convalescent plasma Trx levels (2.5 ng/ml, IQR 0.4–6.9) were not significantly different from those measured during the acute illness. Moreover, convalescent plasma Trx levels were significantly lower than that of healthy controls ($p < 0.001$). By contrast, convalescence plasma IL-1β levels (3.7 ng/ml, IQR 0–25.5) were significantly lower than in acute disease ($p < 0.01$). Likewise, convalescent TNF-α levels (3.7 pg/ml, IQR 0–304.8) were also significantly lower than acute levels ($p = 0.05$). There was no relationship between Trx levels and the concentrations of these pro-inflammatory cytokines either for the population as a whole or in the patients with acute MSS (data not shown).

Sixteen patients with MSS had a predicted mortality greater than 50%. There was no significant correlation between severity of disease and levels of Trx, TNF-α or IL-1β (Table 1). Plasma TNF-α and IL-1β levels were not significantly different between non-survivors ($n = 5$) and survivors ($n = 30$). By contrast, plasma Trx was significantly higher in the small number of non-survivors (36.1 pg/ml, no IQR as $n = 5$) than in survivors (4.0 pg/ml, IQR 0.7–10.2, $p < 0.05$, Table 1).

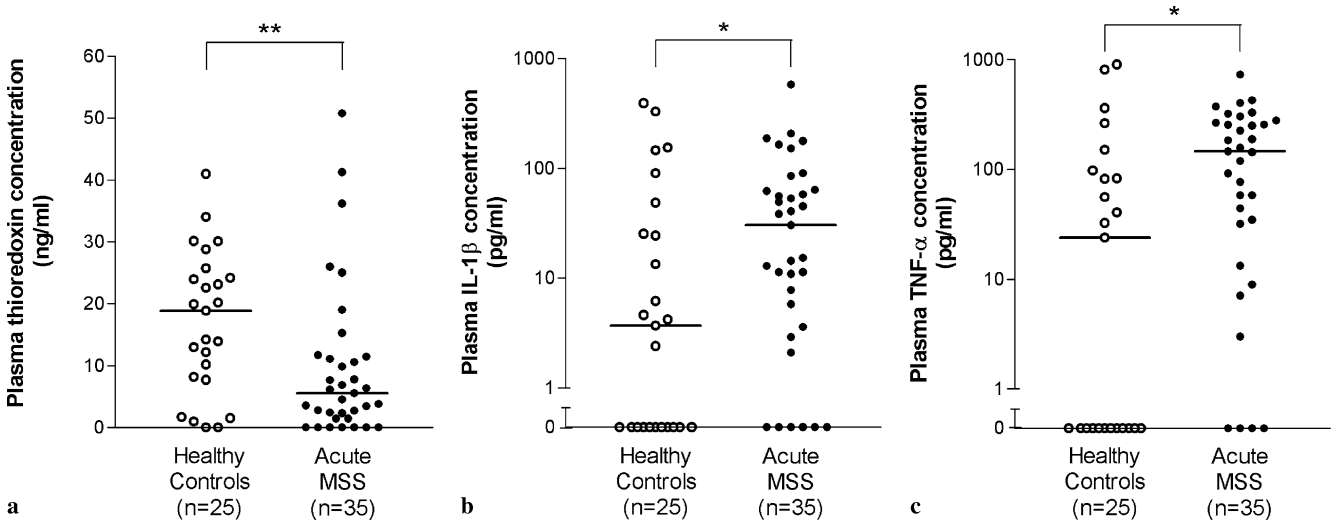

Fig. 1 Concentrations of thioredoxin (**a**), IL-1β (**b**) and TNF-α (**c**) in plasma from healthy paediatric controls (*open circles*) and patients with acute meningococcal septic shock (*MSS*, *closed circles*). Data presented as a scattergram with median values indicated by *horizontal lines*. *$p < 0.05$, **$p < 0.01$

Table 1 Concentrations of thioredoxin, IL-1β and TNF-α in plasma samples from patients with acute meningococcal septic shock categorised according to predicted mortality and survival: median values (*parentheses* interquartile range, where sufficient numbers were present for calculation)

	Thioredoxin (ng/ml)	IL-1β (pg/ml)	TNF-α (pg/ml)
Predicted mortality			
< 50% ($n = 19$)	6.1 (2.3–11.7)	11.3 (2.1–49.4)	91.9 (13.2–267)
> 50% ($n = 16$)	3.7 (0.7–10.5)	58.5 (10.4–164.7)	151.5 (60.4–316)
p[a]	0.540	0.052	0.446
Survival			
Survivors ($n = 30$)	4.0 (0.7–10.2)	22.4 (2.9–60.2)	151.5 (22.5–272.6)
Non-survivors ($n = 5$)	36.1	151.9	119.1
p[a]	< 0.01	0.114	0.525

[a]Mann–Whitney *U*-test

Discussion

Trx is a ubiquitous thiol protein, extracellular levels of which are normally increased in a range of diseases and conditions associated with oxidative stress and inflammation. By contrast, in this study we showed that levels of Trx are lower in plasma from children with MSS during the acute and convalescent phases than in controls. Plasma levels of TNF-α and IL-1β levels were higher in MSS children during acute illness but returned to control levels during convalescence. There was no relationship between either Trx and TNF-α or IL-1β or between levels of Trx, cytokines and markers of predicted mortality. Intriguingly, plasma levels of Trx were significantly higher in, albeit a small group of, non-survivors than in survivors. However, the clinical significance of this finding in such a small population is unclear. One suggestion is that the oxidative stress is so great that anti-oxidant defences are raised despite an inherent defect in the Trx system. Also, the relationship between mortality and plasma levels of TNF-α and IL-1β, previously described by others [9, 10], was not demonstrated in this study again possibly because of the low number of non-surviving children in the study.

This is the first study to report Trx levels in patients with MSS. Previous studies have shown an increase in plasma Trx from control levels of 20–30 ng/ml in healthy adults up to 100 ng/ml Trx in a wide array of conditions associated with oxidative stress and inflammation [4]. Thus our finding of lower plasma Trx levels in MSS patients during the acute and convalescence phase than in controls, was unexpected but in accordance with our previous finding that these patients have compromised anti-oxidant protection [2]. All patients in our study received significant fluid resuscitation (mean 80 ml/kg, range 60–180 ml/kg by the time of sample). However, it is unlikely that haemodilution contributed to a lowering of plasma Trx levels because levels remained low in convalescence. Also, control plasma levels of Trx in healthy children were similar to those in healthy adults, suggesting that Trx levels are not, in general, lower in children.

Several mechanisms might contribute to lower Trx levels in meningococcal disease. First, it is possible that

Trx was more rapidly cleared from the circulation in these patients. However, rates of metabolism in sepsis are usually impaired rather than increased. Second, a decrease in transcription or secretion of Trx might develop in MSS. However, evidence from in vitro studies suggests that oxidative and inflammatory stimuli increase, rather than decrease, Trx expression [11, 12]. Third, there could be an inhibitor of Trx in the plasma of MSS patients. However, our unpublished findings show that addition of MSS plasma to a known concentration of Trx does not alter the Trx concentration, as detected by ELISA (Callister et al. unpublished). This suggests that an endogenous inhibitor of Trx in MSS plasma is unlikely to explain low Trx in MSS. Fourth, a recent study suggested that under oxidizing conditions Trx tends to form large aggregates that are not necessarily detected by ELISA [13]. It is possible that this phenomenon contributed to the apparently low levels of Trx detected by ELISA in plasma of children during the active phase of MSS. However, it is more difficult to explain why the levels remain low in convalescence. Finally, it is possible that some children have a genetic predisposition that results in low levels of Trx. Indeed, the observation that Trx levels in surviving patients ($n = 30$) during convalescence remained significantly lower than controls would support this hypothesis. Previous studies have identified genetic polymorphisms that influence outcome in severe meningococcal disease [14]. Determining the mechanism for persistently low levels of plasma Trx in MSS is the subject of our on-going studies.

In summary, Trx levels were lower in children with MSS during the acute disease and remained lower during convalescence than in controls. This finding contrasts the increase in extracellular Trx levels previously documented in inflammatory/oxidative disorders. Further studies are warranted to determine the nature and significance of the low Trx levels in MSS.

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