Clinical Nutrition xxx (2017) 1–7

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Low serum selenium is associated with the severity of organ failure in critically ill children

M. Broman [a, e], Mattias Lindfors [a, e], Åke Norberg [b, c], Christina Hebert [b], Olav Rooyackers [b, c], Jan Wernerman [b, c], Urban Fläring [d, e, *]

[a] Department of Pediatric Perioperative Medicine and Intensive Care, ECMO-Centre at Astrid Lindgren Children's Hospital, Karolinska University Hospital Stockholm, Sweden
[b] Centre for Surgical Sciences, Karolinska Institutet, Stockholm, Sweden
[c] Department of Perioperative Medicine and Intensive Care, Karolinska University Hospital Stockholm, Sweden
[d] Department of Pediatric Perioperative Medicine and Intensive Care, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Sweden
[e] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 4 October 2016
Accepted 11 June 2017

Keywords:
PICU
Glutathione
Multiple organ failure
Oxidative stress
Selenium concentration in critically ill
Normal selenium concentration

SUMMARY

Background & aims: Low concentration of serum selenium is associated with the inflammatory response and multiple organ failure in adult ICU-patients. Critically ill children are less well characterized. In this study, serum selenium concentration and its possible relation to multiple organ failure as well as glutathione status was investigated in pediatric intensive care (PICU) patients.
Methods: A prospective consecutive cohort of critically ill children (n = 100) admitted to the PICU of a tertiary university hospital, and in addition an age stratified reference group of healthy children (n = 60) were studied. The concentrations of serum selenium and reduced and total glutathione were determined at admission and at day 5 for patients still in the PICU.
Results: Low concentration of serum selenium as well as a high-reduced fraction of glutathione (GSH/tGSH) was associated with multiple organ failure ($p < 0.001$ and $p < 0.01$) respectively. A correlation between low serum selenium concentration and high-reduced fraction of glutathione (GSH/tGSH) was also seen ($r = -0.19$ and $p = 0.03$). The serum selenium concentrations in the pediatric reference group in a selenium poor area were age dependent with lower concentrations in infants as compared to older children ($p < 0.001$).
Conclusions: Both low serum selenium concentration and high reduced fraction of glutathione (GSH/tGSH) were associated with the development of multiple organ failure. The association between low serum selenium concentration and high fraction of reduced glutathione in whole blood favour the hypothesis that selenium is of critical importance for the scavenge capacity of glutathione peroxidase (GPX).

1. Introduction

Critically ill pediatric as well as adult patients with a prolonged stay in the ICU have an increased risk of morbidity and mortality [1,2]. The reason for the poor prognosis is often due to the development of multiple organ failure (MOF) regardless the initial reason for intensive care admission. This can be exemplified by MOF being the most common cause of death in pediatric patients suffering from acute respiratory distress syndrome, not irreversible respiratory failure as might be expected [3]. The mechanisms for the development of MOF are not clarified, but an increased production of reactive oxygen species (ROS) in parallel with an impaired anti-oxidative capacity due to glutathione and selenium depletion has been suggested [4,5]. There are numerous selenoprotein/enzymes involved in various biological functions including maintenance of redox system, especially important in immune competent and thyroid cells [6]. During critical illness, low serum selenium concentrations (S-Se) are seen in both adults and

* Corresponding author. Department of Pediatric Perioperative Medicine and Intensive Care, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Q9:00, 17176 Stockholm, Sweden.
E-mail address: urban.flaring@ki.se (U. Fläring).

http://dx.doi.org/10.1016/j.clnu.2017.06.014
0261-5614/

children, probably due to redistribution from the circulatory compartment to immune cells [7,8]. In unselected adult ICU-patients, conflicting results are seen when selenium supplementation is given [9–11]. However, reduced mortality is reported when adequate selenium supplementation is given to selenium-depleted patients, suggestive of a positive effect [12]. The results might be different depending on if the study was performed in a region with high or low selenium in the soil. In Sweden the S-Se in the adult population is low. For Swedish children, there is no information about S-Se in either health or sickness.

There are only a few reports investigating selenium concentration in critically ill children. The studies consistently show that the S-Se is low in critically ill children as compared to the regionally obtained normal S-Se [8]. Low S-Se has been suggested as a causative factor for idiopathic intractable epilepsy [13]. Also in pediatric patients with burn injuries plasma selenium concentration was reversely related to the numbers of total infections [14]. The shortage of data concerning selenium status in critically ill children and its age distribution, especially in low selenium countries such as Sweden, encouraged us to characterise selenium status in pediatric ICU-patients.

The primary aim of the study was to investigate if low S-Se was associated with the development of multiple organ failure in critically ill children. Secondary aims were (i) to characterise age dependency of the S-Se in children with multiple organ failure (ii) to obtain an age-stratified reference material in healthy children in a selenium poor area; and (iii) to investigate if S-Se was associated with the redox status of whole blood glutathione.

2. Materials and methods

Ethics committee approval was obtained at the Regional Ethical Review Board in Stockholm. Protocol number 2013/2110-31/3, approvals date February 4 (2014). Parents and patients (when possible) were informed orally and in writing before obtaining their informed consent. The protocol was registered at the Australian New Zealand Clinical Trial Registry ACTRN12616001119482.

Patients admitted to the PICU at Astrid Lindgren Children's Hospital at Karolinska University Hospital Stockholm were consecutively screened during four periods between April 2014 and March 2015, covering all seasons of the year. The total time of inclusion was six months.

For the characterisation of an age-stratified reference population of healthy children in a selenium-poor area, pediatric patients undergoing minor surgical procedures in general anaesthesia were included.

The PICU unit is a tertiary pediatric centre in Sweden with mixed surgical (including neurosurgical and thoracic but without cardiac surgery) and medical patients. Inclusion criteria were an expected PICU stay >2 days, sampling and informed consent within 24 h after admission to the PICU. In order to increase the possibility to include patients with MOF, only patients with an expected stay of >2 days were included. Exclusion criteria were failure of sampling, life expectancy less than two days, absence of informed consent at 24 h after admittance, or age >18 years.

Blood sampling for the determination of S-Se and whole blood glutathione concentration as well as routine blood samples needed for organ failure scoring according to the Pediatric logistic organ dysfunction score (PELOD-2 score), Pediatric index of mortality (PIM − 2 score) and organ failure was performed within 24 h after admittance. Serum selenium was determined at admission and at day 5 for those patients still staying in the PICU. The PELOD-2 score quantifies organ dysfunction in six organs: neurologic (Glasgow coma scale and pupillary reaction), respiratory ($PaCO_2$, PaO_2 and whether patient is mechanically ventilated or not), cardiovascular (heart rate and systolic pressure), renal (creatinine), hepatic (glutamic oxaloacetic transaminase and prothrombin time) and hematologic (leukocyte and platelet count), [15]. The most severe value is used in the calculation if a variable is measured more than once during 24 h. The points range from 0 to 6 and depend upon the severity and the organ system. For an organ, the maximal daily score vary from 4 to 6. The maximal number is 33. Numbers of organ failure was determined according to the guidelines and the dysfunction of six organs was evaluated [16]. PIM-2 score is one of the mostly used severity scoring systems to predict outcome in patients admitted to the PICU [17] and the most commonly used score in Europe.

The nutritional routine for the patients was continuous glucose infusion 4–6 mg/kg/min for children <30 kg and 2–4 mg/kg/min if ≥30 kg and start of enteral nutrition according to tolerance using nasogastric tube from day 1. Parenteral nutrition was instituted from day 2, if not full enteral nutrition was expected within 4 days.

Information of the S-Se in healthy children in Sweden and its possible age distribution was not available. Therefore, a reference group consisting of healthy children (n = 60) undergoing minor surgery was assembled for S-Se analyses. The aim was not to recruit a matched control group to the patient cohort. We did not consider it ethical to take blood samples from children without sedation. Blood samples were taken after induction of anaesthesia but before surgery. Examples of the minor procedures were inguinal hernia, removal of orthopaedic pins, minor dermatologic surgery (non-malignant) and orchidopexy procedure. All of the subjects were healthy and well nourished, did not take any medicine or had any chronic disease. The subjects were divided into four different age cohorts (<1, 1–5, 6–12, 13–15 years) with 15 patients in each group.

The impact of comorbidities per se has not been well characterised in pediatric critically ill patients, except being a part of Pediatric index of mortality score. In order to give a reasonable estimate of the comorbidity, we pragmatically chose to use a multispecialty surgical risk score for children which is based upon over 2 millions pediatric surgical admissions (<18 years of age), where 69 comorbidities were scored from 0 to 3 [18]. In our regression calculation, we dichotomised at a total comorbidity score of >1 versus 0–1, where the latter group carried a mortality risk close to zero among the pediatric surgical patients.

Sample handling and analyses of selenium: For each sample, a tube without additive was filled with 1 ml of blood and was left 2 h to clot at room temperature before spinning 10 min at 2000×*g*. After spinning, 300 μL serum was pipetted and then the serum was transferred to an acid washed plastic tube (to avoid contamination) and stored at −80 °C pending analyses. A commercial laboratory company, Australian Laboratory Services (ALS) Scandinavia Luleå determined the analysis of serum selenium concentration using Sector-Field Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [19].

Sample handling and analyses of glutathione: within 2 min, 200 μl of blood was immediately added to 200 μl of 2 mmol phenanthroline in 14% perchloric acid and mixed. Thereafter the sample was frozen in liquid nitrogen, and stored at −80 °C. Pending the analysis, the whole blood samples were treated with three freeze–thaw cycles rapidly in liquid nitrogen before centrifugation at 12500×*g* for 15 min at 4 °C. The supernatant was then used for analyses. HPLC (high-performance liquid chromatography) technique as recently described in full detail [20] was used for the analysis of both reduced and total (after reduction with dithiothreitol) glutathione concentrations in whole blood. In order to achieve the concentration of the oxidized form of glutathione (GSSG), which is a dimer of the reduced form of glutathione, the difference between total and reduced

Please cite this article in press as: Broman M, et al., Low serum selenium is associated with the severity of organ failure in critically ill children, Clinical Nutrition (2017), http://dx.doi.org/10.1016/j.clnu.2017.06.014