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Original article

Erythrocyte selenium concentration as a marker of selenium status☆

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SUMMARY

Background & aims: Plasma selenium concentration and glutathione peroxidase (GPx) activity are commonly used as markers of selenium nutritional status. However, plasma selenium concentrations fall independently of selenium status during the acute phase response and GPx is analytically problematic. The assay for erythrocyte selenium is robust and concentrations are unaffected by the systemic inflammatory response. This study was performed to investigate the validity of erythrocyte selenium measurement in assessing selenium status.

Methods: C-reactive protein (CRP), plasma and erythrocyte selenium concentrations and GPx activity were measured in 96 women from two regions of Malawi with low and high selenium dietary intakes. CRP and plasma and erythrocyte selenium was measured in 91 critically ill patients with a systemic inflammatory response.

Results & conclusions: The median CRP value of all subjects from Malawi was 4.2 mg/L indicating no inflammation. The median CRP value for the critically ill patients was 126 mg/L indicating this group was inflamed. In the non-inflamed population there was a strong positive correlation ($r = 0.95$) between erythrocyte and plasma selenium and a strong positive correlation ($r = 0.77$) between erythrocyte selenium and erythrocyte GPx up to 6.10 nmol/g Hb after which maximal activity was reached. In the inflamed population, plasma selenium was low, erythrocyte selenium was normal and there was a weak correlation ($r = 0.30$) between selenium concentrations in plasma and erythrocytes. This demonstrates that plasma selenium is affected by the inflammatory response while erythrocyte selenium concentration is unaffected and can be used to reliably assess selenium status across a wide range of selenium intakes.

1. Introduction

Selenium is an essential trace element and its importance to human health is well established.[1] Severe endemic selenium deficiency in humans has been linked to Keshan and Kashin–Beck disease. Poor selenium status has also been associated with a number of other health problems and chronic diseases such as cancer,[2] cardiovascular disease,[3] impaired immune function[4] and infertility.[5–7] Conversely, high intakes of selenium have toxic effects and the range in intake between deficiency and toxicity is narrower than for some other trace elements.[8]

Nutritional status of selenium is commonly assessed by direct measurement of its concentration in plasma or indirectly by measuring activity of the selenoprotein glutathione peroxidase (GPx) in erythrocytes (GPx1), plasma (GPx3) and whole blood (GPx1 and 3). However, there are disadvantages with both of these approaches. Problems associated with GPx are typically analytical. In addition, GPx is only useful in assessing selenium status in states of deficiency. Plasma selenium is technically a more robust and standard assay, however, interpretation of results can be difficult in patients with a systemic inflammatory response. It has been observed that there is an inverse relationship between the plasma selenium concentration and the magnitude of the inflammatory response on assessing the plasma CRP concentration.[9,10] This has also been observed in a study using elective knee arthroplasty as a model for the evolution of the systemic inflammatory response.[11]

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Plasma selenium concentrations decreased from pre-operation baseline concentrations in association with a rise in C-reactive protein (CRP) concentrations. This decrease in concentration was transient and concentrations of selenium began to normalise, without selenium supplementation, as CRP concentrations fell. Erythrocyte selenium concentrations were also measured in this study and remained unchanged throughout the development of the systemic inflammatory response indicating that measurement of selenium in this matrix may be a more reliable marker of selenium status in the presence of inflammation.

The aim of this study was to investigate the relationship between erythrocyte selenium concentrations with selenium concentrations in plasma and GPx activity across a wide range of plasma selenium concentrations in subjects who had no evidence of a systemic inflammatory response.

2. Materials and methods

2.1. Subjects

Ninety-six women were recruited from subsistence farming communities split between two regions of Southern Malawi. Eighty of the women were pregnant and in their third trimester. Forty nine subjects (median age 27 years, range 17–38 years) were recruited from Chiradzulu a plateau region near Blantyre and forty seven (median age 27 years, range 16–45 years) from Chikwawa, a flood plain near the Shire river. Venous blood was collected into lithium heparin tubes at the same time of day for each subject.Blood samples were taken as part of a larger study of micronutrient deficiencies in Malawi.[12,13] Ninety-six packed erythrocyte samples were prepared for analysis, however, only ninety-four plasma samples were prepared due to haemolysis in two samples.

To examine the effect of inflammation of selenium concentration in plasma and red blood cells, venous blood samples, were collected from 91 critically ill patients admitted to the intensive care unit (ICU) of the Royal Infirmary, Glasgow, who had respiratory failure requiring ventilatory support, were ≥18 years old, and who had evidence of systemic inflammatory response syndrome as per Bone's criteria.[14] Venous blood samples were collected into lithium heparin tubes on admission to ICU.

2.2. Analytical methods

Samples were centrifuged (500 *g*, 4 °C, 10 min) and the plasma removed for analysis. Packed erythrocytes were prepared by removing any residual plasma and the buffy coat. Samples were stored at −20 °C until analysis. Prior to selenium and iron analysis, packed erythrocytes were digested with 60% nitric acid and diluted 1 in 10 with 25 μg/L germanium, which served as an internal standard, in 1% nitric acid. Selenium and iron were measured simultaneously in plasma and erythrocytes using a 7500 CE inductively-coupled plasma mass spectrometer (Agilent, USA). Erythrocyte selenium was reported as a ratio to haemoglobin concentration to correct for inaccuracies associated with pipetting packed red blood cells. Iron was measured as a surrogate for haemoglobin[11] whose concentration was calculated using the following equation where 64,456 is the molecular weight of haemoglobin in g and the denominator is the number of atoms of iron per haemoglobin molecule.

$$\mathrm{Hb(g/L)} = \frac{\mathrm{Iron(mol/L)} \times 64,456}{4}$$

Activities of GPx1, the predominant form of glutathione peroxidase in erythrocytes, and GPx3 the predominant form in plasma, were measured on a Sapphire 350 spectrophotometer (Audit Diagnostics, Ireland) using a commercially available kit (Randox, Co. Antrim, UK). Haemoglobin was measured colorimetrically on a Sapphire 350 using the Drabkin's method.[15] GPx1 activities were reported as IU/g Hb. Albumin and C-reactive protein were measured in plasma by bromocresol purple and turbidimetric methods respectively using an automated analyzer (Architect; Abbot Diagnosis, Maidenhead, UK).

2.3. Ethics

Procedures were followed in accordance with the Helsinki Declaration of 1975 as revised in 1983.

2.4. Statistics

Data are presented as medians and interquartile ranges. Correlation coefficients (r) and coefficient of determination (r^2) were calculated using Spearman's correlation test. The correlation coefficient was used to measure the strength of linear relationship between all variables with the exception of plasma selenium and albumin where coefficient of determination was used. Medians between groups were tested for statistical significance using the Mann–Whitney U test. A p value of less than 0.05 was used to indicate statistical significance. Statistical analysis was performed using Minitab software (Minitab Inc., USA). Joinpoint regression analysis (version 3.4.3, downloaded from http://srab.cancer.gov/joinpoint/) was performed to investigate the trend in relationship between selenium and GPx.[16,17] The joinpoint selected by the software was used to determine the concentration of selenium at which maximal GPx activity is reached. The correlation coefficients for selenium and GPx were then calculated for selenium concentrations below the selected joinpoint.

3. Results

The median CRP value of all subjects from Malawi was 4.2 mg/L (interquartile range = 1.7–9.0 mg/L) indicating that subjects did not have an inflammatory response (<10 mg/L). No significant difference was found in CRP values between regions (median 3.1 mg/L, interquartile range = 1.2–8.9 mg/L vs median 5.2 mg/L, interquartile range 2.1–9.1 mg/L, $p = 0.078$). Plasma selenium concentrations were significantly lower ($p < 0.001$) in individuals from the plateau region (median = 0.85 μmol/L, interquartile range = 0.67–0.96 μmol/L) compared to those in the flood plain region (median = 2.42 μmol/L, interquartile range = 1.84–3.01 μmol/L) (Fig. 1). Erythrocyte selenium concentrations were also significantly lower ($p < 0.001$) in individuals from the plateau (median = 3.75 nmol/g Hb, interquartile range = 2.79–4.71 nmol/g Hb) compared to those in the flood plain (median = 14.53 nmol/g Hb, interquartile range = 10.31–20.13 nmol/g Hb) (Fig. 2). There was a strongly significant correlation between plasma selenium and erythrocyte selenium concentrations ($r = 0.95$, $p < 0.001$) (Fig. 3).

Joinpoint analysis of the relationship of plasma selenium concentration with GPx1 activity (Fig. 4), erythrocyte selenium concentration with GPx1 activity (Fig. 5), and plasma selenium concentration with GPx3 activity (Fig. 6) estimated that the "best fit" model was a single joinpoint with the data divided into two separate linear segments. The first linear segment represented an increase in GPx activity with selenium concentration; there were strong, significant correlations of GPx1 activity with plasma selenium concentration ($r = 0.77$, $p < 0.001$), GPx1 activity with erythrocyte selenium concentration ($r = 0.75$, $p < 0.001$), and GPx3 activity with plasma selenium concentration ($r = 0.58$, $p < 0.001$). The second linear segment represented a plateau at which GPx activity reached a maximum in relation to selenium concentration.