Brief Communication

Lower plasma levels of selenium and glutathione in smear-positive tuberculosis patients in Malawi

Arntsen A[1], Sakhi AK[2], Kalfoss T[3], Maleta K[4], Blomhoff R[2], Duttaroy AK[2], Bjune AG[1]

Abstract

Objective: The objective of this study was to investigate selenium and glutathione levels in pulmonary tuberculosis patients and controls in Malawi.

Methods: The case-control study included 19 patients and 15 apparently healthy controls for whom, levels of selenium and glutathione were measured.

Results: The plasma selenium levels were significantly lower in tuberculosis patients compared with the controls (p = 0.02).

Conclusion: No significant differences were observed for the plasma glutathione levels (p = 0.39). [*Ethiop. J. Health Dev.* 2011;25;(3):230-232]

Background

Both protein-energy malnutrition and deficiencies in micronutrients are associated with a significant impairment of various interactions and functions of the cell-mediated immune system (1). A study in Malawi showed that selenium deficiency occurred in 87 % of tuberculosis (TB) patients (2) while a study in Ethiopia, on antioxidants status in untreated TB patients, showed that the patients were deficient in glutathione (GSH) compared to healthy controls (3). Selenium via selenoproteins and GSH are important endogenous antioxidants that protect against oxidative damage and regulate immune functions (4, 5). The objective of this study was thus to investigate plasma levels of selenium and GSH in pulmonary TB patients and controls in Malawi.

Methods

A case-control study was conducted in Malawi between September and December 2006. Fifty-four patients were asked to participate of whom 42 accepted. Fifteen patients did not have follow-up samples, 6 withdrew the consent without any reason and data from 2 patients was not included due to inappropriate sample collection. For the controls, 47 were asked to participate and 25 consented. Six controls did not have follow–up samples and 4 withdrew the consent without any reason. The final sample set consisted of 19 patients and 15 controls aged 15-55 years.

TB was confirmed when a patient was smear-positive on at least two of three sputum specimens. The study was done 2-8 weeks after onset of treatment because then they had resumed their ordinary diet. Patients with a previous history of TB and extra pulmonary TB patients were excluded. Controls were randomly selected according to the "random-walk method" (6). Matching of age and sex was done and adjusted for the statistical analysis.

Ethical clearance was obtained from the Regional Committee for Medical Research Ethics, Norway and the Malawi College of Medicine Research Ethics Committee, Malawi. Participants were enrolled after signing a written informed consent form.

Statistical analysis was done using SPSS for Windows 14.0 (Statistical Package for the Social Sciences, Chicago, IL, USA)

Procedure for Blood Testing

Seven ml blood was drawn into tubes containing heparin from non-fasting participants. Two ml blood was then transferred to an eppendorf vial containing 50 µl 2.0 M serine borate buffer for glutathione measurements. Blood samples were protected from bright light.

The samples were stored for 40-43 minutes and then centrifuged in Labofuge 200 (Osterode, Germany) at 2500 g for 10 minutes. All samples were frozen at -20 ºC for 3 weeks and then at -80 ºC until transferred on dry ice to Norway for analysis. The procedure for GSH analysis has been described by Bohn et al. (7). Selenium was measured with PerkinElmer, (Elan® DRC™ II Inductively Coupled Plasma Mass Spectrometry instrument, Concord, Canada).

Results

Characteristics of study participants are shown in table 1. Adjusting for age and sex, TB patients had significantly lower levels of selenium compared to the controls (p = *0.02).* No significant difference was observed for GSH (p = 0.39) (Table 1).

[1]Institute of General Practice and Community Medicine, University of Oslo, Norway, Heidi Arntsen Pastor Fangens vei 20 A 0854 OSLO, NORWAY, Mobile telephone: 0047 959 07 396, E-mail: heidi.arntsen@gmail.com;
[2]Dept. of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway;
[3]Fürst Medical Laboratories, Oslo, Norway;
[4]College of Medicine, University of Malawi

Table 1: **Characteristics of the study group, mean values of Selenium and GSH levels in Patients and controls** and multivariate linear regression analysis in both groups***

	TB patients n = 19 n (%)	**Controls n = 15 n (%)**	**Coefficient**	**95% CI**	**P-value**
Women	6	5			
Men	13	10			
Age, years, mean [SD]	28.0±7.0	32.1±10.5			
Illiterate	5 (26)	5 (33)			
Farmers	14 (74)	12 (80)			
Body Mass Index, mean [SD]	18.5±2.0	21.3±1.9			0.80
Selenium** (µmol/L)	1.10±0.22	1.23±0.19			0.10
GSH** (µmol/L)	3.85±1.68	4.48 ±1.52			0.29
Group* (selenium)			-0.17	-0.31-0.02	0.02
Group * (GSH)			-0.53	-1.81-0.73	0.39

**Mean±Standard deviation [SD] GSH=Glutathione
*Adjusted for age and sex. CI= Confidence Interval

Discussion

After adjusting for age and sex, selenium concentration in TB patients was 0.22 µmol/L lower than the controls (p = 0.02). The difference is significant and in agreement with Kassu et al. (8). The concentration of GSH in TB patients was 0.53 µmol/L lower than the controls (p = 0.39). This result is not in agreement with results found in Ethiopia (3) where the GSH concentrations were significantly lower in TB patients. The possible explanations could be the degradation of GSH by enzyme gamma-glutamyl transferase during sample collection and preparation (9), small sample size and selection bias. We have avoided degradation of GSH by using serine borate buffer that inhibits enzyme gamma-glutamyl transferase (9).

Selenium is a trace mineral, involved in several key metabolic activities via selenoproteins. These enzymes are essential to protect against oxidative damage and to regulate immune functions (4). GSH is an antioxidant and protects cells from toxic effects of reactive oxygen species. It plays an important role in regulating the cellular immunity and protects against mycobacteria (5). The reason for lower selenium and GSH levels in TB patients is unclear, but could be explained by the increased oxidative stress and the reduced levels of antioxidant capacity found in this group (3). Other factors like variation in diet, absorption, the severity of the disease and being HIV positive may also have influenced the plasma levels of selenium and GSH.

Limitations of the study were the small sample size and no statistical correction for HIV status in both groups. Further studies are hence needed to understand the observed deficiencies of selenium and GSH in TB patients.

Conclusion

This study showed that TB patients had lower plasma levels of selenium compared with controls.

Acknowledgements

The authors wish to thank F. Eick, the staff at Mangochi District Hospital and St. Martins Hospital for their cooperation and contributions. We thank the Department of Nutrition and Fürst Medical Laboratories for the laboratory analyses. We also wish to thank the University of Oslo and the Juul Christiansen Foundation for financial support.

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