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Serum Protein thiol status in Pregnant women with Malaria

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ABSTRACT

Oxidative stress is thought to be involved in the pathophysiology of malaria, especially in pregnancy, where natural resistance is markedly reduced. One of the major antioxidant both in intracellular and extracellular body fluids is reduced thiol (-SH) groups existing as protein bound thiols. The current study is designed to know the levels of such protein bound thiols in serum and urine of pregnant women with malaria. The study was conducted on urine and serum samples of 30 normal pregnant women and 30 pregnant women with malaria. Levels of serum and urine protein thiols were determined by spectrophotometric methods using dithionitrobenzoic acid (DTNB). Serum albumin, urine protein were estimated by semiauto analyzer (ERBA CHEM-7). There was significant decrease in serum albumin ($p < 0.01$) and serum protein thiols ($p < 0.01$) in pregnant women with malaria compared to normal pregnant women. In urine samples there was significant increase in protein ($p < 0.01$) and decrease in protein bound thiols ($p < 0.01$) in pregnant women with malaria compared to normal pregnant women. Serum albumin correlated positively with serum protein thiol ($r = 0.561$), ($p < 0.01$) and urine protein thiol ($r = -0.410$), ($p < 0.01$) correlated negatively with urine protein thiols. Protein bound thiols, the major antioxidants in the body, are decreased in pregnant women with malaria. Due to increased consumption of protein bound thiols in such oxidative environment, there was significant decrease in protein bound thiols in urine.

Keywords: Urine thiols, Serum thiols, pregnant women with malaria with malaria.

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INTRODUCTION

Malaria is a parasitic disease affecting red blood cells that is transmitted by female mosquitoes of the *Anopheles* genus. Following infection by sporozoites found in the mosquito's salivary glands, the host liver cells are initially used by the parasites to grow and multiply. Parasites released into the circulation subsequently infect red blood cells, proliferate in them and eventually cause them to burst. Of the four parasitic protozoa causing malaria, *Plasmodium falciparum*, *P. Vivax*, *P. mlariae* and *P.ovale*. *Plasmodium falciparum* is the most dangerous, causing between 700,000 and 2.7 million deaths annually, most of which are in children and pregnant mothers [1-3]. Oxidative stress plays an important role in the development of malarial anemia [4, 5]. Malarial infection activates the immune system of the body thereby causing the release of reactive oxygen species (ROS). The malaria parasite itself generates large quantities of reactive oxygen species and also through its interaction with phagocytes [6-8].

Oxidative stress can produce profound alterations to cellular membrane lipids, proteins, and nucleic acids, impairing cell metabolism and viability, and it is thought to be involved in aging [9] and diseases such as diabetes mellitus, uremia, atherosclerosis, rheumatoid arthritis, adult respiratory distress syndrome, reoxygenation injury, HIV infection, and cystic fibrosis. Oxidative stress corresponds to an imbalance between the production of reactive oxygen species, mainly the superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($\cdot OH$), peroxy radicals ($LOO\cdot$), and hydrogen peroxide (H_2O_2), and protective mechanisms. Several enzymatic systems can detoxify reactive oxygen species: superoxide dismutase catalyzes the conversion of $O_2^{\cdot-}$ to H_2O_2 and works concomitantly with catalases and a selenoprotein, glutathione peroxidase. The concentration of reduced glutathione (GSH) is a limiting factor in this enzymatic process, which requires the maintenance of a high reduced-to-oxidized glutathione ratio as achieved by glutathione reductase. In addition, some reducing agents, such as GSH, vitamin E, vitamin C, and β -carotene, act as free radical scavengers to nonenzymatically detoxify reactive oxygen species.

The tripeptide glutathione (g-l-glutamyl-l-cysteinylglycine) is the major intracellular non protein thiol compound, and it plays a major role in the protection of cells and tissue structures from oxidative injury. Glutathione can be reduced (GSH), oxidized, or bound to proteins (protein bound thiols) play a major role in maintaining the antioxidant status of the body. The thiols are the major antioxidants in body fluids which are known to reduce highly reactive free radicals thus protecting the biomolecules. Such thiol groups have been studied and determined in different disease conditions and found to be decreased in different diseases compared to healthy controls [10, 11]. In non-pregnant individuals, abnormal total protein excretion is typically defined as greater than 150 mg daily. In normal pregnancy, urinary protein excretion increases substantially, due to a combination of increased glomerular filtration rate and increased permeability of the glomerular basement membrane [12]. Total protein excretion is considered abnormal in pregnant women when it exceeds 300 mg/24 hours [13]. The current study was designed to know the levels of protein bound thiols in such oxidative environment

and to establish relation between serum albumin and protein thiols with protein bound thiols in urine of malaria patients with pregnancy.

Subjects

The study was conducted on urine and serum samples of normal pregnant women (n = 30) and pregnant women with malaria (n = 30). This study was approved by institutional review board and informed consent was obtained from all subjects involved in the study. Under aseptic conditions blood samples were collected in plain vacutainers from ante-cubital veins of normal pregnant women and pregnant women with malaria. Blood was centrifuged at 2000 g for 15 minutes at 4°C for clear separation of serum and assay was performed immediately. Random urine samples were collected and assayed for urine thiols.

Reagents and Methods

Special chemical 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), was obtained from Sigma chemicals, St Louis, MO, USA. All other reagents were of analytical grade.

Serum and urine thiols were measured by a spectrophotometric method using DTNB [14, 15]. Briefly, 900 µL of 0.2 M Na₂HPO₄ containing 2 Mm Na₂EDTA, 100 µL serum or urine and 20µL of 10Mm DTNB in 0.2M Na₂HPO₄ were taken in an Eppendorf tube and warmed to 37°C. The solution was mixed in a vortex mixer and transferred to a cuvettes, and the increase in absorbance was measured at the end of 5 min at 412 nm in Systronics 118 UV spectrophotometer. Appropriate sample and reagent blanks were prepared and the corrected absorbance values were used to calculate the concentration of thiols using calibration curve. Values were expressed in µmoles/L for serum and urine thiols.

Statistical analysis:

The results were expressed as mean ± standard deviation (SD). A p value of <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-17, Chicago, USA). An independent t test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

RESULTS

There was significant decrease in serum albumin (p<0.01) and serum protein thiols (p<0.01) in pregnant women with malaria as compared to normal pregnant women. In urine samples, there was significant increase in protein (p<0.01) and decrease in protein bound thiols (p<0.01) in pregnant women with malaria as compared to normal pregnant women (table 1). Serum albumin correlated positively with serum protein thiols (fig 1) (r = 0.561, p<0.001) and urine protein correlated negatively with urine protein thiols (fig 2) (r = -0.410, p<0.001).

Table 1: Demographic characteristics and biochemical parameters of pregnant women with malaria patients and normal pregnant women (expressed in mean \pm SD)

	Normal pregnant women (n = 30)	Pregnant women with malaria patients (n = 30)
Gestational age	27-40 weeks	27-40 weeks
Serum Albumin(g/dl)	3.6 \pm 0.3	2.8 \pm 0.2
Serum thiols (μ moles/L)	192.3 \pm 12.6	110.4 \pm 6.2*
Urine protein(mg/dl)	178.2 \pm 25.42	289.05 \pm 56.4*
Serum thiols (μ moles/L)	250.9 \pm 62.2	173.9 \pm 51.3*
Urine thiols (μ moles/L)	68.8 \pm 40.1	20.12 \pm 28.1

*P value < 0.01 compared to normal pregnant women

Figure 1: Correlation between serum albumin and serum protein thiols.

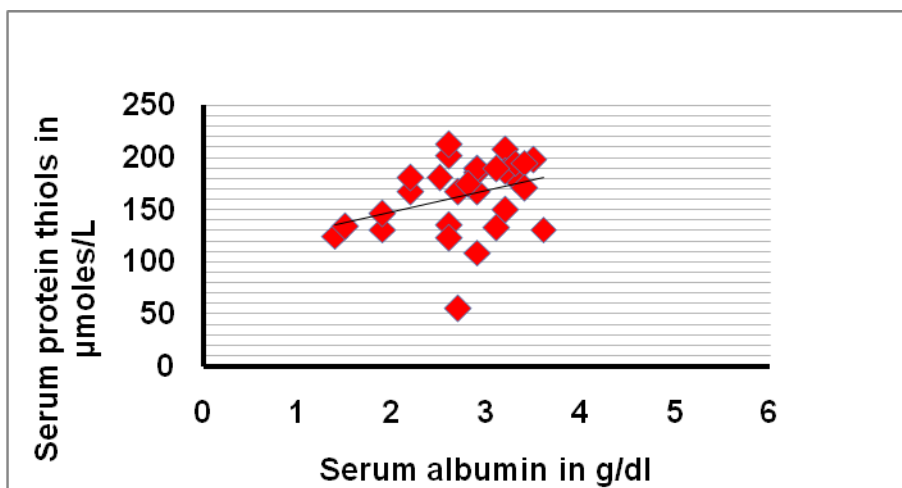
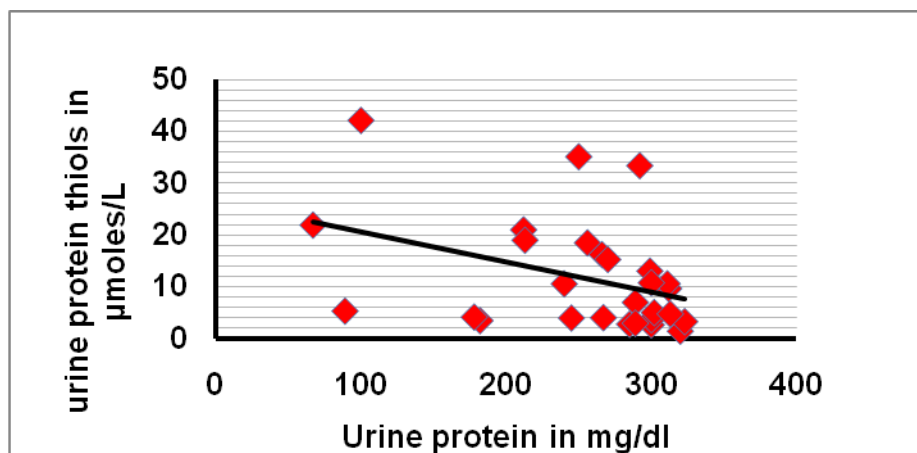


Figure 2: Correlation between urine proteins and urine protein thiols.



DISCUSSION

The results presented in this study demonstrated that the concentration of sulphhydryl groups (-SH) or groups existing as protein bound thiols in serum were markedly reduced in pregnant women with malaria compared to normal pregnant women. Existence of oxidative stress in pregnant women with malaria is well proved [16-18]. Decreased -SH levels may be due to enhanced free radical generation in pregnant women with malaria. These reduced thiol groups were oxidized by electron deficient free radicals, in the process there occurs oxidation of -SH groups present over plasma proteins, majority of them over albumin, and glutathione. Since -SH groups are the major antioxidants that contribute to the antioxidant pool of the body fluids, hence oxidation of such -SH groups can significantly contribute to the oxidative damage to biomolecules in pregnant women with malaria.

We speculate that the decreased thiols in urine of pregnant women with malaria could be because of increased oxidation of -SH groups in serum due to already existing oxidative stress. Depletion of -SH groups by oxidative free radicals may be the possible cause for decreased levels of urine -SH levels seen in our study in pregnant women with malaria, although other possible causes cannot be ruled out.

Increase in oxidative stress in pregnant women with malaria compared to normal pregnant women, might have occurred as a result of toxic effects of upsurge reactive oxygen species produced by immune system as well as synchronized released of O₂⁻ during hemoglobin degradation by malaria parasite. Our observation of significant positive correlation of serum albumin with serum protein thiols and negative correlation of urinary protein with urine protein thiols levels, possibly indicates increased free radical generation by malaria infection in pregnancy and thus increased consumption of reduced -SH groups.

In conclusion, our data suggests that increased consumption of serum protein bound thiols in pregnant women with malaria may be the possible biochemical basis for decrease in protein bound urinary thiols.

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