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Plasma glutathione and oxidized glutathione level, glutathione/oxidized glutathione ratio, and albumin concentration in complicated and uncomplicated falciparum malaria

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ABSTRACT

Objective: To compare the level of glutathione (GSH) and oxidized glutathione (GSSG), the ratio of GSH/GSSG and the concentration of albumin in plasma of patients with complicated and un-complicated falciparum malaria.

Methods: This research was a cross sectional study using comparison analysis with the plasma GSH and GSSG, the ratio of plasma GSH/GSSG and the concentration of plasma albumin as variables. The complicated malaria patients were obtained from Dr. Saiful Anwar Hospital Malang, whereas uncomplicated malaria patients were obtained from the Regency of Pleihari South Kalimantan. Plasma GSH and GSSG levels were determined by the spectrophotometer at the wave length of 412 nm, whereas the concentration of albumin was determined by bromocresol green method in the pH of 4.1.

Results: There were no significant differences between the level of plasma GSH and GSSG in complicated and uncomplicated malaria patients, as well as the ratio of plasma GSH/GSSG in the two groups ($P = 0.373$; $P = 0.538$; and $P = 0.615$, respectively, independent *t*-test). In contrast, the plasma albumin concentration in complicated malaria patients were significantly higher than uncomplicated malaria patients ($P = 0.000$, Mann Whitney *U* test).

Conclusions: It can be concluded that the average of plasma GSH and GSSG level, also plasma GSH/GSSG ratio in complicated malaria are not different from uncomplicated malaria. Although plasma concentration of albumin in both groups is below the normal range, there is an increase in complicated malaria that might be as compensation of oxidative stress.

1. Introduction

The main sources of oxidant during malaria infection are from hemoglobin digestion in the food vacuole of parasite inside host erythrocyte, the synthesis and folding of proteins within the endoplasmic reticulum, as well as the production of the energy (adenosine triphosphate) in the mitochondria. However, parasites maintain the redox equilibrium using antioxidant systems [1]. Oxidative stress can be regarded as an imbalance between oxidant production and antioxidant defenses. Overproduction of oxidant or reactive oxygen species can be toxic to cells causing oxidation of macromolecules, including lipids, protein and nucleic acids followed by cellular and tissue damage [2].

Glutathione (GSH) is the most abundant antioxidant in all aerobic cells, presenting with high-concentrations in body fluids and tissue. GSH which is synthesized from L-glutamate, L-cysteine and L-glycine is critical for protecting the tissue from oxidative stress, acting as a free radical scavenger and inhibitor

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The study protocol was performed according to the Helsinki declaration and approved by the committees of research of the Faculty of Medicine, Universitas Brawijaya. Informed written consent was obtained from the patients.

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of lipid peroxidation [3]. Most of free radical forms are removed by GSH using enzymatic reduction, whereas the elimination of H_2O_2 requires enzymatic catalysis by GSH peroxidase and catalase. During this process GSH becomes oxidized glutathione (GSSG). This GSSG characterized by a disulfide bond between two molecules of GSH is efficiently reduced back to GSH by the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent catalysis of the flavoenzyme GSH reductase [4].

When a strong oxidative stress is exposed to mammalian cells, this condition may require not only enhanced GSH action to maintain redox status, but also augmented energy supply and precursors to enhance GSH content and transport it to the places where it is needed. Moreover, when oxidative stress becomes prolonged and cellular systems are no more able to counteract the abundant of oxidative stress, the ratio of GSH/GSSG will reduce as a consequence of decreased amount of free GSH [4]. The ratio of reduced GSH to GSSG can be used as an indicator of cellular health. Measuring the ratio of GSH/GSSG in pathological tissues and experimental models compared to those in normal tissues is an excellent procedure to know the efficacy of antioxidant therapeutics in maintaining cellular redox [3].

The invasion of malaria parasite in human erythrocytes causes a potential oxidant that will induce the antioxidant resistance of erythrocytes [5]. The GSH metabolism of the parasites is regulated by its biosynthesis, reduction and efflux. GSH biosynthesis in *Plasmodium falciparum* is related to the rapid efflux of GSH from the infected erythrocytes and the parasite's inability to scavenge sufficient amounts of the tripeptide from its environment to compensate that efflux [6]. GSH efflux from *Plasmodium falciparum* infected erythrocytes is actually greater than that from uninfected erythrocytes [7].

Albumin, a non-glycosylated, negatively charged plasma protein, with ascribed ligand-binding normally accounts for over 50% of total plasma protein content. Albumin is present as transport protein and serves multifunctional activities like antioxidant functions, and enzymatic activities. Albumin could potentially reduce oxidant effects through scavenging antioxidant actions, modifying redox balance, and regulating cell signaling moieties active in mediating pro-inflammatory response [8,9]. A previous study has shown that albumin resuscitation may reduce mortality rate in children with severe malaria. Mortality decreased in children receiving albumin than in those treated with gelofusine (Fisher's exact test, $P = 0.06$). The effect of albumin on mortality showed a pooled relative risk of death with albumin administration of 0.19 (95% *CI*: 0.06–0.59; $P = 0.004$) compared to other fluid boluses [10].

Based on the above facts and theories, this study will compare GSH and GSSG level, GSH/GSSG ratio and albumin concentration in plasma between complicated and uncomplicated falciparum malaria.

2. Materials and methods

2.1. Sample collection

This study was a cross sectional study with descriptive analysis data to determine the level of GSH, GSSG and the ratio of GSH/GSSG and the level of albumin in the plasma of malaria patients. This research was conducted at the Central Laboratory of Biomedical, Faculty of Medicine Universitas Brawijaya and the Central Laboratory of Dr. Saiful Anwar Hospital Malang. The experiment was conducted from November 2009 to April 2010. Ethical clearance was provided on 16 November 2009 by the committees of research of the Faculty of Medicine, Universitas Brawijaya.

The research subject was blood plasma of complicated malaria falciparum patients ($n = 9$) from Dr. Saiful Anwar Hospital Malang (most of them were imported cases from Kalimantan island) and uncomplicated malaria falciparum patients ($n = 10$) from the district of South Kalimantan, Pleihari. Blood was taken with the informed consent. All blood chemical parameters [urea, creatinine, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and bilirubin level] were determined in Central Laboratory of Dr. Saiful Anwar Hospital Malang.

Reduced GSH and GSSG level were detected using the Oxis kit/Research TM. Catalog number 21040. The level of the disulfide GSSG formed consisting of two molecules of GSH through a combination of the reaction between GSH and 5,5′-dithio-bis (2-nitrobenzoic acid) to form 5-thio-2-nitrobenzoic acid and GS-5-thio-2-nitrobenzoic acid (glutathione adduct of GSH), which immediately reduced to GSH. The ratio of GSH/GSSG was then counted by dividing the difference level between the GSHt and GSSG (reduced GSH) by the level of GSSG or (GSHt−2 GSSG)/GSSG. Albumin concentration in plasma was measured by bromocresol green method in Roche/Hitachi Cobas c 501-analyzer.

2.2. Preparing GSH samples

Fifty microliters of blood plasma was inserted into the base of microcentrifuge tube then frozen at −70 °C. In the experiment day, samples were thawed and immediately mixed. A total of 350 μL of 5% metha phosporic acid (MPA) was inserted into the tube (dilution 1/8 of the original sample). Samples were vortexed for 15–20 s and then centrifuged at 3 500 r/min for 10 min. Then 50 μL of MPA extract was added to 3 mL of assay buffer (1/61 dilution acid supernatant). Supernatant was placed in a cooler for measuring in spectrophotometer.

2.3. Preparing GSSG samples

Ten microliters of 1-methyl-2-vinyl trivate pyridium were put into the tube then 100 μL blood plasma was carefully inserted to the bottom of centrifuge tube. The tube was mixed gently. The samples were frozen at −70 °C. In the experiment day samples were thawed then incubated at room temperature for 2–10 min. A total of 290 μL of 5% MPA was inserted into a chilled tube containing the samples (1/4 dilution of the original sample). Samples were vortexed for 15–20 s and centrifuged at 3 500 r/min speed for 10 min. Then 50 μL MPA extract was added to 700 μL of GSSG buffer (1/5 dilution acid supernatant). Supernatant was placed in a cooler for measuring in spectrophotometry (dilute 1/60).

2.4. Determination of levels of GSH, GSSG, and the ratio of GSH/GSSG

A total of 200 μL of standards and samples were added to the cuvettes. Then 200 μL of chromogen was added to each cuvette, and 200 μL of the enzyme was added to each cuvette, mixed and then incubated at room temperature for 5 min. A total of 200 μL