



## **Protection of Renal Function ByHyperbaric OxygenDuring Plasmodium berghei ANKA Infection**

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**Abstract : Background:**Malaria cases have consistently increased and the number of deaths remains largely unchanged.Malaria associated renal injury has a high level of morbidity and mortality. High level of blood urea nitrogen / BUN and plasma creatinine is one of the major factors associated with mortality in humans infected with malaria, indicating that impairment of renal function. HBO is widely used as an adjunctive therapy for many diseases, it is known that HBO hasan action as antiplasmodium, antiinflammation and antioxidant effects.

**Objective:** The aim of this study was to determine the effect of HBO on BUN and creatinine levels in rats infected with *P. berghei* ANKA.

**Methods:**This research was conducted experimentally post-test only control group on six groups of rats. The samples used were 24 male Rattus norvegicus wistar strain that have been infected by *Plasmodium berghei*ANKA and divided into 6 groups. Group 1 was given combination artesunate and 1, 5 ATA HBO, grup 2 was given combination artesunate and 3, 0 ATA HBO, group 3 was given 1, 5 ATA HBO, group 4 was given 3, 0 ATA HBO, group 5 was given artesunate and group 6 was given aquadest. HBO therapy is carried out for 10 days and the observation of the levels of BUN and creatinine in mice after treatment on tenth day.

**Results:**Descriptive analysis and statistical analysis (Kruskal wallis snd Mann whitney U posthoc) showed a significant difference ( $p < \alpha = 0.05$ ) on mean levels of BUN and creatinine group receiving combination of artesunate and HBO 3,0 ATA compared to other groups.Hyperbaric oxygen has the effect of reducing the levels of BUN and creatinine in rats infected by *P.berghei* ANKA.

**Keywords :** HBO, *P.berghei* ANKA, malaria , BUN and Creatinine.

### **Introduction**

Malaria was global problem of life-threatening public health through the bite of Anopheles female mosquito infected with *Plasmodium* sp. Almost half of the world's population were at risk of contracting malaria especially in high-risk groups of infants, toddlers and pregnant women<sup>1,2</sup>.There were5 species of *Plasmodium* causes malaria in humans, *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*,

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*Plasmodium vivax* and *Plasmodium knowlesi*. *P.falciparum* and *P. vivax* were the 2 most common species of malaria, with the most pathogenic *P. falciparum*<sup>1,3</sup>. Among the five species of parasites that can infect humans, *Plasmodium falciparum* is the most severe form leading to the development of cerebral damage (cerebral malaria), respiratory distress, and renal injury.

The life cycle of malaria in the human body consists of an exoerythrocytic cycle that occurs in the liver and the erythrocytic cycle that occurs in the circulation. The blood stage parasite infects the erythrocyte responsible for all of the symptoms and pathology associated with malaria. Malaria associated renal injury occurs between 1 and 4% of hospitalized adult with a mortality that can reach up to 45%. The pathogenesis of malaria associated renal injury is multifactorial and not well characterized, but hypothesis suggests the involvement of oxidative stress during malaria infection and damage to the vital organs especially renal organ. It has been proposed that renal injury also depends on the cytoadherence of infected erythrocytes to the microvasculature and the host immune response, which play a role in tubular and glomerular damage, respectively. For screening of renal function, increasing of blood urea nitrogen (BUN) and creatinine in plasma can be used as critical markers. High level of plasma creatinine is one of the major factors associated with mortality in humans infected with *Plasmodium falciparum*, indicating that impairment of renal function is a great risk factor for these patients infected with malaria<sup>4,5</sup>.

Hyperbaric oxygen / HBO is a systemic action of administering 100 % oxygen in a closed room with a pressure of more than 1 atmosphere / above sea level atmospheric pressure<sup>6,7</sup>. Previous studies about administration of HBO to malaria were very limited, because there was only 1 study that examined the use of HBO against cerebral malaria and the rest was only an article review. Research conducted by Blanco et. al (2008) used HBO 3 ATA dose for 1 hour continuously against C57BL / 6 mice which were inoculated by *P.berghei* ANKA which is one model of cerebral malaria mice. The results of this study indicate that OHB can prolong the life ability of mice infected with malaria. Administration of HBO can reduce the excessive inflammatory response by decreasing proinflammatory cytokines and also significantly decreasing the sequestration of immune cells in brain tissue. Histopathological examination of brain tissue shows that administration of HBO improves the blood brain barrier (BBB), so it is concluded that OHB can prevent death from cerebral malaria and has the potential as adjuvant therapy of cerebral malaria<sup>8</sup>. In addition, HBO is widely used as an adjunctive therapy for many conditions, such as diabetic ulcer healing, traumatic brain injury, and ischemic stroke. Based on the background above, this study aims to observe effect of HBO on BUN and creatinine levels in animal models (male *Rattus norvegicus* wistar strain) infected by *Plasmodium berghei* ANKA.

## Experimental

This research was a true laboratory experimental research and the data in this research was taken only once at the end of the research so that the research design used was Post test Only Randomized Control Group Design. The population in this study were male *Rattus norvegicus* obtained from Faculty of Veterinary Medicine of Airlangga University Surabaya. *Plasmodium berghei* ANKA (PbA) strain that will be inoculated in experimental animals was obtained from the Faculty of Medicine Brawijaya University. The inoculation process is carried out at Faculty of Veterinary Medicine of Airlangga University Surabaya. Administration of hyperbaric oxygen therapy in the animal hyperbaric chamber was carried out at the Navy's Institute of Marine Health (LAKESLA) Naval Hospital (RSAL) of Ramelan Surabaya. The ethical feasibility test in this study was obtained from Research Commission of University of Hang Tuah University of Surabaya.

The samples on this research were male *Rattus norvegicus* wistar strain aged 10 - 12 weeks ranged from 100 - 150 grams. Male rats were selected for the reason that their biological condition is stable when compared to female mice whose biological condition is affected by the estrous cycle. Adaptation to the environment was carried out for 7 days in all groups of rats and maintained its condition, food and drink. Animal models placed in a plastic tub of size 30 x 20 cm and given chaff. Each tub contains 4 rats. All animals were allocated to metabolic cages and kept in an air-conditioned environment (22– 24 °C) with 12 hours alternating periods of light/dark and free access to food and fresh water<sup>9,10,11</sup>.

The sample size used for each treatment group was calculated based on the Federer WT (1967) formula  $(k-1)(n-1) \geq 15^{12}$ , the sample size used for each group was at least 6 rats and sample size used in the whole group is 24 samples of rats. In the study used 6 groups : (1) The first group consisted of 4 rats (*Rattus norvegicus*) wistar strain infected with PbA and obtained artesunate and HBO 1.5 ATA for 2 hours for 10

consecutive days<sup>13</sup>; (2) The second group consisted 4 rats (*Rattus norvegicus*) wistar strain infected with PbA and obtained artesunate and HBO3 ATA for 2 hours for 10 consecutive days<sup>13</sup>; (3) The third group consisted of 4 rats (*Rattus norvegicus*) wistar strain infected with PbA and obtained HBO 1.5 ATA for 2 hours for 10 consecutive days.; (4) The fourth group consisted of 4 rats (*Rattus norvegicus*) wistar strain infected with PbA and obtained HBO3 ATA for 2 hours for 10 consecutive days; (5) The fifth group consisted of 4 rats (*Rattus norvegicus*) wistar strain infected with PbA obtained artesunate; (6) The sixth group consisted of 4 rats (*Rattus norvegicus*) wistar strain infected with PbA obtained standart feed and aquadest.

The artesunate tablet solution was made with distilled water (1 g/mL) and administered to the animals by orogastric tube for a period of three days. The dosage of artesunate was 4 mg/Kg body weight daily for 3 days. All the animals were weighed before the experiment

*Plasmodium berghei* ANKA was *Plasmodium* which can cause malaria in rodents. and it has similar biological nature and sensitivity to drugs with malaria species that infect humans, especially *P. falciparum*. The blood stage forms of both parasites were stored in liquid nitrogen after in vivo passages in animal models. Blood donor mice that have been calculated percent of parasitemia reach  $\geq 20\%$  and diluted with PBS, taken as much as 200  $\mu$ l to intraperitoneally infected to all groups of research. Infected rats were marked and put in a cage according to the treatment group. Naive rats were infected intraperitoneally (i.p.) with  $10^6$  infected red blood cells (iRBC) and parasitemia level were monitored daily<sup>8,14,15</sup>.

Groups 1 – 4 of PbA infected mice were exposed daily to 100% oxygen at a pressure of 1.5 and 3.0 atmospheres (ATA) for 2 hours per day in animal chamber of Navy's Institute of Marine Health (LAKESLA) Naval Hospital (RSAL) of Ramelan Surabaya from day 1 to 10 post infection (10 day exposure). Animals were anesthetized with ketamine (83 mg/g) and xylazine (13 mg/g) administered intraperitoneally for all of the blood drawing procedures including tail blood samples.

Parasitemia was determined daily in all infected groups. The blood sample was picked from the mice tail blood and placed on a clean slide and a thin smear was made, the smear was fixed with methanol and stained with Giemsa stain for a few minutes and washed off under a running tap water and left to air dry for about 25 minutes and then viewed under a microscope at x400 magnification<sup>16</sup>.

Screening of renal function, increasing of blood urea nitrogen (BUN) and creatinine in plasma. On the tenth day blood was taken in the heart of the rat, before blood was taken, the rat was anesthetized using ketamin injection. Blood was put into a tube that had been given EDTA and examined using ABX micros 2000 at RSAL clinical pathology laboratory from Ramelan Surabaya.

The one way ANOVA was used to analyze and compare the results at a 95% confidence level if normality and homogeneity obtain. If the normality test of the data is not normally distributed or in the homogeneity test the data is not homogeneous, the data can not be analyzed with One way ANNOVA test, but with non parametric Kruskal Wallis test. If the Kruskal-Wallis test yields  $p < 0.05$  (there is a significant difference), then the Mann-Whitney U test is followed. Values of  $p < 0.05$  were considered significant.

## Results

### Blood Urea Nitrogen/BUN Levels

**Table 1. : Examination of Blood Urea Nitrogen/BUN Levels**

Groups	Minimum	Maximum	Mean	Std. Deviation
G 1	13,00	16,00	15,00	1,41
G 2	8,00	13,00	11,00	2,16
G 3	17,00	20,00	18,75	1,26
G 4	22,00	30,00	25,75	3,86
G 5	17,00	18,00	17,75	0,50
G 6	22,00	28,00	24,75	2,75

Note : G1 : Group receiving artesunate and 1.5 ATA hyperbaric oxygen; G2: Group receiving artesunate and 3 ATA hyperbaric oxygen; G3: Group receiving 1.5 ATA hyperbaric oxygen ; G4: Group receiving 3 ATA hyperbaric oxygen; G5 : Group receiving artesunate; G6: Group receiving aquadest; unit of BUN (mg/dL)

Normality test results indicate that the normality requirement is not fulfilled because  $p < 0,05$ . This study was using non parametric analysis Kruskal Wallis to analyze the difference of BUN levels between the groups. Statistical analysis showed that there is a significant difference which  $p$  value is  $0.001 < \alpha$  ( $\alpha = 0.05$ ) on BUN level between all groups research (figure.1). Descriptive analysis of BUN levels showed that the highest BUN level in G4 (25,75 +3,86) and the lowest is G2 (11.00 + 2.16)(table 1). Post hoc analysis showed that BUN levels between groups ( G1 with G2 – G6; G2 with G3 –G6; G3 with G4 and G6; G4 with G5; G5 with G6) was significantly different ( $p < 0.05$ ), but there are no significant differences between groups G3 with G5; G4 with G6 ( $p > 0,05$ ). Based on post hoc analysis, we can conclude that administration of artesunate and 3,0 ATA hyperbaric oxygen can decrease BUN level on this study.

### Creatinine Level

**Table 2 : Examination of Creatinine Level**

Groups	Minimum	Maximum	Mean	Std. Deviation
G 1	0,60	0,80	0,68	0,10
G 2	0,30	0,70	0,53	0,17
G 3	1,30	1,70	1,50	0,18
G 4	1,50	1,70	1,60	0,08
G 5	0,70	1,30	1,08	0,26
G 6	1,70	2,50	2,05	0,34
p value is (0.001) < $\alpha$ ( $\alpha = 0.05$ )				

Note : G1 : Group receiving artesunate and 1.5 ATA hyperbaric oxygen; G2: Group receiving artesunate and 3 ATA hyperbaric oxygen; G3: Group receiving 1.5 ATA hyperbaric oxygen ; G4: Group receiving 3 ATA hyperbaric oxygen; G5 : Group receiving artesunate; G6: Group receiving aquadest; unit of creatinine (mg/dL)

Normality test results indicate that the normality requirement is not fulfilled because  $p < 0,05$ . This study was using non parametric analysis Kruskal Wallis to analyze the difference of creatinine level between the groups. Statistical analysis showed that there is a significant difference which  $p$  value is  $0.001 < \alpha$  ( $\alpha = 0.05$ ) on creatinine level between groups research (figure 2). Descriptive analysis of creatinine level showed that the highest creatinine mean level in G6(2,05+0,34) and the lowest mean level is G2 (0,53 +0,17) (table 2). Post hoc analysis showed that creatinine mean levels between groups ( G1 with G2 – G4 and G6; G2 with G3 –G6; G3 with G5-G6; G4 with G5- G6; G5 with G6) was significantly different ( $p < 0.05$ ), but there are no significant differences between groups G1 with G5; G3 with G4 ( $p > 0,05$ ). Based on post hoc analysis, we can conclude that administration of artesunate and 3,0 ATA hyperbaric oxygen can decrease creatinine level on this study.

### Discussion

Kidney or renal function tests are common laboratory tests used to evaluate how well the renal are working. Such tests include: blood urea nitrogen (BUN) and serum creatinine test. Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body. Serum creatinine is an important indicator of renal health because it is an easily measured by product of muscle metabolism excreted unchanged by the renal. It is synthesized by the liver from the methylation of glycoamine by S-adenosyl methionine. It is then transported through the blood to the other organs, muscles, and brain where it is phosphorylated to phosphocreatine. During this reaction, creatine and phosphocreatine are catalyzed by creatine kinase, and a spontaneous conversion to creatinine may occur. Creatinine is removed from the blood by the renal through glomerular filtration and also by proximal tubular secretion. There is little or no tubular reabsorption of creatinine. When its filtration in the renal is impaired, blood creatinine levels rise and so its levels in blood and urine may be used to calculate the creatinine clearance. Creatinine levels in the blood and urine correlates with the glomerular filtration rate (GFR) while blood creatinine levels alone are used to calculate the estimated GFR<sup>5,17</sup>.

Renal injury related malaria was confirmed by the increase of plasma BUN and creatinine levels. It can be described that malaria associated renal injury is proposed to be a consequence of parasite adhesion and exacerbated immune response against oxidative stress products during infection. Therefore, proinflammatory

molecules, hypoxia and products of oxidative stress have a central role to the development of the pathogenesis of malaria associated renal injury. The extent of reactive oxygen species-induced oxidative damage can be exacerbated by decreased efficiency of antioxidant and cytoprotective defense mechanisms. Moreover, modifications in the permeability of renal vascular endothelium and sequestration of infected erythrocytes to the vascular endothelium decreased O<sub>2</sub> delivery to cells and tissues and contributed to increased hypoxic microenvironments<sup>4,18,19</sup>.

In this study, we provide evidence that describes the effects of HBO to reduce BUN and creatinine levels in plasma during PbANKA infection. The BUN and creatinine levels in plasma showed approximately significant increase, but HBO inhibited this increase. Hence, HBO had a positive effect on plasma BUN and creatinine abnormalities. Administration of HBO can increase partial oxygen pressure in plasma. Hyperbaric oxygen delivery increases the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to hyperoxia. ROS and RNS produced from HBO administration can be a signaling molecules that regulate inflammatory transcription factors, so that the production of proinflammatory cytokines can be controlled. Increased ROS and RNS can also activate antioxidant enzymes. Regulation of the inflammatory process, increasing of oxygen and activation of antioxidants may be a mechanism for repairing ischemic tissue damage. Previous studies that showed similar results to this study were Edremittlioglu *et al.* (2005) study showed that HBO treatment has a beneficial effect on renal dysfunction in sepsis. The probable reason for this effect is the reduction in oxidative damage because of the increase in antioxidative capacity<sup>20</sup>. Berkovitch *et al.* (2014) study conclude that HBO is safe in healthy rats, regarding renal function<sup>21</sup>. Contrary to the results of this study, Berkovitch *et al.* (2017) study conclude that treatment of gentamicin-induced nephrotoxicity with either HBOT or 100% oxygen for a consecutive 5 days had no beneficial renal effect, and mortality was observed only in the HBOT group<sup>22</sup>.

## Conclusions

Renal injury related malaria was confirmed by the increase of plasma BUN and creatinine levels. Combination of artesunate and hyperbaric oxygen has the effect of reducing the levels of BUN and creatinine in rats infected by *P. berghei* ANKA

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