DIFFERENCES OF TUMOR NECROSIS FACTOR ALPHA (TNF-α) PLASMA CONCENTRATION IN MALARIA PATIENTS WITH ANEMIA AND WITHOUT ANEMIA

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ABSTRACT

Anemia is an important complication of malaria. The pathogenesis of malaria anemia is not well understood. Some inflammatory cytokines such as TNF-α are believed to be involved in the pathogenesis of malaria anemia because concentration of plasma TNF-α was increased in malaria patients with anemia. The aim of this study was to compare the concentration of plasma TNF-α in malaria patients with anemia and without anemia. Concentration of plasma TNF-α in 20 malaria patients with anemia and 20 plasma samples malaria patients without anemia from patients at Tanjung and Gangga primary health centers, west Lombok during December 2007 until June 2008 was measured using ELISA. The results were analyzed using 2 sample t test. The average concentration of plasma TNF-α in falciparum malaria patients with anemia 145.27 ± 24.18 pg/ml while the average concentration of plasma TNF-α in falciparum malaria patients without anemia 36.26 ± 16.18 pg/ml. The average concentration of plasma TNF-α in vivax malaria patients with anemia 97.83 ± 13.96 pg/ml while the average concentration of plasma TNF-α in vivax malaria patients without anemia 30.41 ± 14.57 pg/ml. We conclude that TNF-α concentration in malaria patients with anemia is significantly higher than the TNF-α concentration in malaria patients without anemia (p = 0.000...).

Keywords : *Falciparum malaria, vivax malaria, Anemia, TNF-α, ELISA*

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INTRODUCTION

Malaria is one of infectious diseases become a major health problem in the world today other than Tuberculosis (TB) and infection of Human Immunodeficiency Virus (HIV). More than 100 countries around the world, especially in tropical and subtropical countries (40% of world population) face the problem of infection by malaria. Until recently malaria was still a threat, often leading to death if not treated properly. World Health Organization (WHO) estimates that one billion cases of malaria occurred each year with a mortality of 1.5 to 3 million people per year (White & Breman 2004).

The latest data states that malaria ranked number ten causes of morbidity and dropped to number five out of six infectious disease was the cause of death in the world (Jacobson 2004). Household Health Survey (Household Health Survey) in 2001 showed that malaria ranked number eight out of 10 major disease leading cause of death in Indonesia. Number of malaria cases in Indonesia annually approximately 15 million and the number of deaths with a mortality rate of 35 000 urban and rural 0.7% 1.7% (Consensus treatment of malaria 2003).

Lombok Island, where the execution of this research is the development area and a relatively new tourism destination, has been declared as areas of malaria hypo-mesoendemik since several years ago with a spleen rate and parasite rate of 8-26% (Dikes Province NTB 2006). The number of Malaria patients in West Lombok regency in the year 2007 is fairly high, as many as 2056 patients with *P. falciparum* infection in 1450 patients, 591 patients *P. vivax*, a mix between *P. falciparum* and *P. malariae P. vivax* 10 patients and five patients (Dikes Lobar 2007).

Research in Africa shows approximately 60% of people affected by the bite of Anopheles mosquitoes infected with Plasmodium will, half of those infected will provide clinical symptoms of malaria (paroxysmal and periodic fever, chills, headache, accompanied by the presence or absence of anemia and enlarged spleen) and only about 2% manifests as severe malaria (Greeworld 1999).

Other studies in Africa showed five children who suffer from malaria or the complications of one of them died and others suffered from anemia, low birth weight (LBW), epilepsy and neurological disease (Snow RW et al. 2004). Research in Samarinda showed approximately 50% of people infected with Plasmodium will provide clinical symptoms of malaria such as anemia (Carta 2005). Erythrocyte invasion of Plasmodium that cause damage in erythrocytes and hemolysis is a major cause of anemia in malaria is well on mild malaria and severe malaria (White & Ho 1992)

Anemia in malaria is one important complication because it can cause problems in daily life such as decreased physical capacity, damage or secondary organ dysfunction such as cardiac arrhythmias and congestive heart failure. Anemia in children causes growth retardation and motor and mental development of the child. In children aged 11-15 years in the development of emotional problems, general health status, daily habits and disturbances of concentration and memory.

Parents who suffer from anemia can cause problems such as heart attack / heart failure, and anemia 13% of individuals who can develop into cancer in 10 years time period compared with individuals who are not anemic (5%) (Menendez et al. 2000). TNF-α is a cytokine Pro inflammation that play a role in various infectious and inflammatory diseases (Abbas & HA Litchtman 2003). Some literature states that TNF-α plays an important role in the pathogenesis of malaria. This can be proved the existence of symptoms that resemble symptoms of malaria due to endotoxaemia, TNF-α to be the main mediator in the onset of symptoms such as fever, anemia, impaired liver function, renal, pulmonary edema (ARDS = adult respiratory distress syndrome), decreased blood pressure until shock as well as cerebral malaria (Abbas et al. 2000).

Examination of TNF-α level in malaria infection can be used to differentiate *P. falciparum* and *P. vivax*, besides used as a prognostic in cases of severe malaria (Angulo & Fresno 2002, Abrams et al. 2005). TNF-α levels can be used as a diagnostic tool to infectious Plasmodium (Abrams et al. 2005). Mean levels of TNF-α in *P. falciparum* was significantly higher than *P. vivax*. This was caused by differences in ability to stimulate TNF-α production of Plasmodium species is the second antigen (Breitling 2006).

There is a correlation between cerebral malaria and other forms of severe malaria (severe anemia, hypoglycemia, renal failure, pulmonary edema, shock, DIC, hemoglobinuria) with TNF-α levels are higher in the plasma of patients with infection *P. falciparum* (Hidajati 2005). In anemia due to malaria, TNF-α is a cytokine that causes bone marrow suppression by stimulating production of oxygen radicals, besides TNF-α also increase otokrin eritrofagositosis and stimulate differentiation of stem cells (stem cell) by shifting toward monosoit eritropoietik system.

TNF-α, IL-2, IFN-γ increase in iron uptake into cells of macrophages, increased expression of transferrin receptors and increased synthesis of ferritin in liver cells. These factors also led to a damning hipoferemia anemia in chronic diseases (Weis et al. 1995). TNF-α levels will be increased in patients with anemia of malaria (Steketee 2001).

The absence of a full description of elevated levels of TNF-α plasma from malaria patients which results in anemia, the researchers were interested and motivated to carry out research on the differences in plasma levels of TNF-α between malaria patients with anemia and malaria patients without anemia.

MATERIALS AND METHODS

This research is observational research with cross sectional design. The population are all malaria patients with anemia at Puskesmas and Puskesmas Tanjung Barat Lombok Ganga and all malaria patients without anemia at Puskesmas and Puskesmas Tanjung Barat Lombok Ganga Samples from malaria patients in each group were taken randomly (simple random). Patients who meet the admission criteria of the sample were asked their consent to participate in research. Subsequently 2 ml of blood drawn for complete blood hemoglobin level and especially for the inspection of four ml TNF-α.

Previous inspection has been conducted at the beginning of the ICT malaria diagnosis malaria. Place of sampling are conducted at both Puskesmas Tanjung and Puskesmas Barat Lombok Ganga inpatient, outpatient and clinic assistants. Laboratory research conducted at Department/Clinical Pathology Dr.Soetomo Hospital R & D section and biomedical Research Unit Mataram General Hospital - NTB. Research will be conducted over six months ie December 2007 to June 2008.

RESULTS

Based on the research that has been done at Puskesmas and Puskesmas Tanjung Barat Lombok Ganga got 88 malaria patients with the following details falciparum malaria patients were 45 (52.9%) patients, patients with vivax malaria in 39 (45.9%) patients and patients with mixed falciparum malaria and vivax malaria as much as one (1.2%) patients. Of the 45 falciparum malaria patients who meet the criteria for receiving the sample of 20 patients, divided into two groups, whereas from 39 patients with vivax malaria who meet the admission criteria for the sample of 20 patients, divided into two groups: patients with anemia of malaria and malaria sufferers group without anemia.

The sample of falciparum malaria patients with anemia patients numbered 10, consisting of seven male patients and three female patients, with age ranged from 18 to 45

years and the mean age of 30.50 ± 9.87 years, while the range of 7.3 g of hemoglobin/dl to 10.8 g/dl with a mean of 9.52 ± 1.11 hemoglobin g/dl. Sample characteristics falciparum malaria patients with anemia can be seen in table 1.

The sample of falciparum malaria patients without anemia patients numbered 10, consisting of four male patients and six female patients, with age ranged from 18 to 50 years and the mean age of 34.20 ± 10.82 years, while the range of hemoglobin concentration 11.9 g/dl to 16.2 g/dl with a mean hemoglobin level 13.78 ± 1.28 g/dl. Sample characteristics falciparum malaria patients without anemia can be seen in Table 1.

Table 1. Characteristic of the general subject of research falciparum malaria patients with anemia and without anemia in Puskesmas and Puskesmas Tanjung Lombok Barat months Ganga March 2008

Characteristic	*Falciparum* malaria patients with anemia	*Falciparum* malaria patients without anemia
Total	10	10
Sex		
- Male	7 (70%)	4 (40%)
- Female	3 (30%)	6 (60%)
Age (years)		
- Mean	30.50	34.20
- SD	9.87	10.82
- Range	18 – 45	18 – 50
Hb concentration (g/dl)		
- Mean	9.52	13.8
- SD	1.11	1.8
- Range	7.3 – 10.8	11.9 – 16.2

Sample vivax malaria patients with anemia patients numbered 10, consisting of six male patients, and four female patients, with an age range from 19 to 50 years and the mean age of 30.30 ± 9.96 years, while the range of 8.9 g of hemoglobin / dl to 10.8 g / dl with a mean hemoglobin concentration of 10.05 ± 0.60 g / dl. Sample characteristics vivax malaria patients with anemia can be seen in table 2.

Sample vivax malaria patients without anemia patients numbered 10, consisting of eight male patients and two female patients, with age ranged from 17 to 51 years and the mean age of 30.20 ± 12.07 years, while the range of hemoglobin concentration 11.7 g / dl to 15.3 g / dl with a mean hemoglobin concentration of 13.54 ± 1.18 g / dl.

Table 2. Characteristic of the general subject of the study patients with vivax malaria with anemia and without anemia in Cape Community Health Center and Health Center Gangga Lombok Barat bulan Maret 2008 Ganga West Lombok in March 2008

Characteristic	*Falciparum* malaria patients with anemia	*Falciparum* malaria patients without anemia
Total	10	10
Sex		
- Male	6 (60%)	8 (80%)
- Female	4 (40%)	2 (20%)
Age (years)		
- Mean	30,30	30,20
- SD	9,96	12,07
- Range	19 – 50	17 – 51
Hb conctentration (g/dl)		
- Mean	10.05	13.54
- SD	0.60	1.18
- Range	8.9 – 10.8	11.7 – 15.3

TNF-α standard curve

Standard curve made in advance prior to TNF-α ELISA test to study subjects. ELISA test was performed on standard solutions in order to understand the value of absorbance of standard solution, which has been carried out serial dilutions to obtain seven types of TNF-α levels are 6000 pg/ml, 2000 pg/ml, 666.7 pg/ml, 222.2 pg/ml, 74.07 pg/ml, 24.69 pg/ml, 8.23 pg/ml. Sample diluents solution used as a zero standard solution with the levels of TNF-α 0 pg/ml. The reading of the results carried out with a micro ELISA reader at a wavelength of 450 nm. Absorbance of standard solution of TNF-α can be seen in table 2.

Table 3. TNF-α absorbance values of serial dilutions of TNF-α standard solution

Standard solution	TNF-α concentration (pg/ml)	Absorbanc (ג = 450 nm)
0	0	0.055
1	8.23	0.102
2	24.69	0.124
3	74.07	0.164
4	222.2	0.302
5	666.7	0.673
6	2000	1.348
7	6000	1.873

TNF-α levels of the sample is calculated using a standard curve of assistance that can be seen in Figure 1. The value of absorbance of the samples obtained and then the horizontal line is drawn up to cut the standard curve. Through the intersection of vertical lines are then drawn toward the axis X, so that can be detected sample levels of TNF-α.

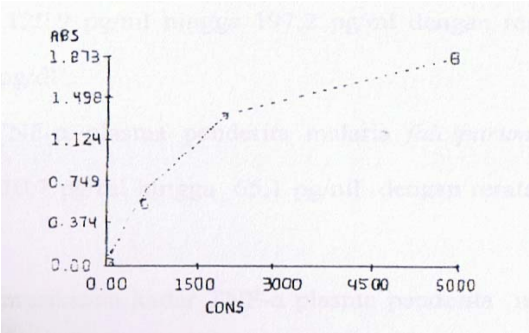

Figure 1. TNF-α standard curve

Strengthening the quality inspection of TNF-α

Strengthening the quality inspection of TNF-α by finding imprecision. Difference absorption in each duplicate sample is calculated to obtain the standard deviation (SD) or coefficient of variation (CV). Imprecision calculated from examination of 10 samples taken in duplicate. SD in this study amounted to 0.014 pg/ml, while the CV in this study amounted to 8.9%.

Strengthening the quality of the ICT Malaria

Strengthening the quality of ICT malaria has been included in the examination procedure ICT malaria. The emergence of a line on the control area (C) shows that the ICT malaria can be used (ACON 2004). The assessment of plasma levels of TNF-α falciparum malaria patient with anemia and without anemia Plasma levels of TNF-α falciparum malaria patient with anemia

ranged from 120.9 pg/ml to 197.2 pg/ml with average of 145.27 pg/ml and SD 24.18 pg/dl. Plasma levels of TNF-α falciparum malaria patients without anemia ranged from 10.7 pg / ml to 65.1 pg / ml with a mean of 36.26 pg / ml and SD 16.18 pg/dl. The assessment of plasma levels of TNF-α falciparum malaria patient with anemia and without anemia can be seen in table 4 below.

Table 4. Levels of TNF-α falciparum malaria patient with anemia and without anemia

Falciparum malaria patient with anemia			*Falciparum* malaria patient without anemia		
No	Absorbance	TNF-α concentration (pg/ml)	No	Absorbance	TNF-α concentration (pg/ml)
1	0.206	120.9	1	0.105	10.7
2	0.208	122.4	2	0.115	20.4
3	0.210	124.1	3	0.118	20.7
4	0.213	131.5	4	0.125	30.2
5	0.215	132.9	5	0.130	36.4
6	0.218	144.8	6	0.132	41.3
7	0.226	155.2	7	0.135	42.6
8	0.235	159.6	8	0.138	44.5
9	0.246	164.1	9	0.143	50.7
10	0.275	197.2	10	0.158	65.1
		Mean : 145.27 SD: 24.18			Mean : 36.26 SD : 16.18

Data plasma levels of TNF-α between falciparum malaria patients with anemia and without anemia test for normality with Kolmogorov-Smirnov test. Results of normality tests on *P. falciparum*-infected patients with anemia is p = 0.618. In falciparum malaria patients without anemia is p =0.417. Based on the results of normality test, the two groups had a normal distribution (p> 0.05).

Results of analysis of two independent samples t test showed that the plasma levels of TNF-α among patients with anemia of falciparum malaria and falciparum malaria patients without anemia had a significant difference (p = 0.000 ...). Plasma levels of TNF-α falciparum malaria patients with anemia (145.27 ± 24.18 pg / ml) was significantly higher compared to patients falciparum malaria without anemia (36.26 ± 16.18 pg / ml).

The assessment of plasma levels of TNF-α vivax malaria patients with anemia and without anemia

Plasma levels of TNF-α vivax malaria patients with anemia ranged from 120.9 pg / ml to 197.2 pg / ml with a mean of 145.27 pg / ml and SD 24.18 pg / dl. Plasma levels of TNF-α vivax malaria patients without anemia ranged from 10.7 pg / ml to 65.1 pg / ml with a mean of 36.26 pg / ml and SD 16.18 pg / dl. The assessment of plasma levels of TNF-α vivax malaria patient with anemia and without anemia can be seen in table 5 below. Data plasma levels of TNF-α among patients with vivax malaria with anemia and without anemia test for normality with Kolmogorov-Smirnov test. Results of normality test in patients with anemia were infected *P. vivax* p = 0.309.

In vivax malaria patients without anemia is p = 0.806 . Based on the results of normality test, then both groups have a normal distribution (p> 0.05). Results of analysis of two independent samples t test showed that the plasma levels of TNF-α among patients with anemia and malaria vivax falciparum malaria patients without anemia had a significant difference (p = 0.000 ...).

Plasma levels of TNF-α vivax malaria patients with anemia (97.83 ± 13.96 pg / ml) was significantly higher compared to patients vivax malaria without anemia (30.41 ± 14.57 pg / ml).

DISCUSSIONS

In Indonesia, malaria is spread across the island to the degree of endemism that differ and can be contagious in the area with an altitude up to 1800 meters above sea level (Rampengan TH, 2002). Malaria-endemic areas, especially the eastern part of Indonesia, including East

Kalimantan and West Nusa Tenggara, while the regions with the highest endemicity is the province of Papua, Maluku, East Nusa Tenggara.

Table 5. Levels of plasma TNF-α vivax malaria patients with anemia and without anemia

Falciparum malaria patient with anemia			*Falciparum* malaria patient without anemia		
No	Absorbance	TNF-α concentration (pg/ml)	No	Absorbance	TNF-α concentration (pg/ml)
1	0.168	76.6	1	0.040	15.7
2	0.175	83.9	2	0.086	18.9
3	0.178	88.4	3	0.112	20.1
4	0.181	90.6	4	0.115	20.4
5	0.184	96.1	5	0.120	23.1
6	0.187	98.4	6	0.122	24.6
7	0.203	103.2	7	0.126	30.6
8	0.204	105.4	8	0.137	43.9
9	0.206	111.6	9	0.142	49.6
10	0.210	124.1	10	0.150	57.2
		Mean: 97.83 SD: 13.96			Mean: 30.41 SD : 14.57

Recently reported some of the focus of a new malaria in areas previously free of malaria on the island of Java and Sumatra (re-emerging infectious disease) (Laihad 2003).

Malaria is a disease caused by the malaria parasites (Plasmodium) that go into the human body in the form of asexually transmitted by female Anopheles mosquitoes. Most species are found in approximately 95% of the world is *P. falciparum* and *P. vivax* (CDC, 2007). Prevalence of Plasmodium species in an area can usually be associated with the tendency of the majority of clinical malaria in the area, with its complications of severe malaria is only found in patients with falciparum malaria (Bradley 1995).

Research in Africa shows approximately 60% of the population infected with *P. falciparum*, half of those infected will provide clinical symptoms of malaria (paroxysmal and periodic fever, chills, headache, accompanied by the presence or absence of anemia and enlarged spleen) and only about 2% manifested as severe malaria (Greeworld 1999). Other studies in Africa showed five children who suffer from malaria or the complications of one of them died and others suffered from anemia, low birth weight (LBW), epilepsy and neurological disease (Snow et al. 2004, Hellen et al. 2004). Research in Samarinda results shows approximately 50% of people infected with *P. falciparum* will provide clinical symptoms of malaria such as anemia (Carta 2005).

Differences between plasma levels of TNF-α falciparum malaria patient with anemia and without anemia of falciparum malaria patients all data obtained from measurements in both groups were tested for normality prior data with Kolmogorov-Smirnov test. Results of normality test showed that both groups have a normal distribution (p> 0.05). The result of two independent samples t test that showed significant, meaning there is a difference between plasma levels of TNF-α falciparum malaria patients with anemia and falciparum malaria patients without anemia (p = 0.000 ...).

Test results also showed that plasma levels of TNF-α falciparum malaria patients with anemia was significantly higher (145.27 ± 24.18 pg / ml) than plasma levels of TNF-α falciparum malaria-infected patients without anemia (36.26 ± 16 .18 pg / ml). Research by Fernandez et al. (2008) different from the results of this research. TNF-α levels in patients without anemia of falciparum malaria is significantly higher than falciparum malaria patients with anemia (p = 0.396)

As stated by Harpaz on studies of several volunteers, individuals who are not immunized her levels of TNF-α can increase the time given malaria antigen, whereas in individuals previously immunized with TNF-α levels showed a stable trend in low grade (Harpaz et al. 1992). According to Riley (1999), T cells that have been exposed to malaria antigens have the effect of potentiation of TNF-α production by macrophages in

malaria patients with the mediation of IFN-γ produced by these T cells.

Chizzolini, et al. (1990) suggests that CD4 + T-cells from patients with acute malaria are still vulnerable (new malaria infections experienced 1-2 times in recent months), when stimulated with malarial antigen in vitro, will produce levels of IFN- γ that is higher than T cells from individuals who have been immune to malaria (eg. African population living in high endemic areas with stable transmission since childhood).

So could be concluded that the production of TNF-α in individuals with non-immune and semi-immune individuals will be higher than that already immune. Malaria parasite has a complex life cycle stages are marked by the emergence of morphology and different characteristics. Malaria parasite has a complex life cycle stages are marked by the emergence of morphology and different characteristics. This parasite also has a large genetic diversity and may experience relatively rapid antigenic variation. (Conway 1997, Sutherland 1998). (Conway 1997, Sutherland 1998). Therefore, residents of endemic regions acquire immunity is very slow.

Immunity can only be obtained with repeated exposure and continuous, and a solid immunity is also difficult to obtain because of this immunity was short-lived, usually obtained only non-sterilizing immunity, in which individuals no longer show clinical symptoms but still contain parasites although in small amounts (Mohan and Stevenson, 1998). Lombok Island and Brazil declared a malaria area hypo-mesoendemik, whose inhabitants acquire immunity is very slow (non-immune status or semi-immune) to obtain levels of TNF-α are higher in patients with falciparum malaria.

One cause of anemia in malaria is the imbalance of Th1 and Th2 cytokines role in malaria patients with anemia. Excess cytokines from T helper cell type 1 (Th1) (TNF-α, IFN-γ) and nitric oxide (NO) has implications in the pathogenesis of cerebral malaria, is also in the mechanism of bone marrow suppression, and eritrofagositosis diseritropoisis visible on malaria with anemia. High levels of Th2 cytokines (IL-10, IL-12) that can neutralize a high Th1 cytokines and may prevent the development of severe malaria anemia (Ian et al. 2004).

IL-10 can inhibit the synthesis of several cytokines that are stimulated by macrophages, NK cells T lymphocytes, suppress Delayed-type hypersensitivity responses, stimulate proliferation and differentiation of B cells into antibody-producing cells, and inhibit the synthesis of several cytokines that stimulated macrophages after binding to cellular receptors 110 kd (Conti 2003, Opal 2000).

In falciparum malaria, there was sequestration of red blood cells infected in the bone marrow, also saw an increase in macrophages in between erythroblast, which phagocyte malaria pigment. Sequestration is to increase levels of TNF-α in bone marrow causing bone marrow suppression activities to eritropoisis causing anemia in malaria (Schlichterle et al. 1996) TNF-α levels of falciparum malaria patients with anemia was lower in the study Fernandez, et al (2008) compared to other studies.

This is due, among others, the role of IL-10 which inhibits the synthesis of TNF-α to TNF-α production decreased. People with malaria have of the inhibition of TNF-α production and the possibility of production and accumulation of TNF-α concentration in the organs, so TNF-α levels in organs is higher than the levels in the systemic circulation. Several studies have reported that malaria patients who have antibodies against malaria antigens indicate levels of TNF-α is smaller. (Harpaz et al.1992).

Differences between plasma levels of TNF-α vivax malaria patients with anemia and vivax malaria patients without anemia

Data obtained from measurements in both groups were tested for normality prior data with Kolmogorov-Smirnov test. Results of normality test showed that both groups have normal distributions ($p > 0.05$). The result of two independent samples t test that showed significant, meaning there is a difference between plasma levels of TNF-α vivax malaria patients with anemia and vivax malaria patients without anemia ($p = 0.000$ ...).Test results also showed that plasma levels of TNF-α vivax malaria patients with anemia was significantly higher (97.83 ± 13.96 pg / ml) than plasma levels of TNF-α vivax malaria-infected patients without anemia (30.41 ± 14 , 57 pg / ml).

The study by Fernandez et al (2008) different from the results of this study. Levels of TNF-α in patients with vivax malaria without anemia was significantly higher compared with vivax malaria patients with anemia ($p = 0.01$).There is no explanation about the levels of TNF-α in patients with vivax malaria without anemia was higher than vivax malaria patients with anemia.

Differences between plasma levels of TNF-α falciparum malaria patients with anemia and vivax malaria patients with anemia

The mean plasma levels of TNF-α falciparum malaria patients with anemia (145.27 ± 24.18 pg / ml) higher than the mean plasma levels of TNF-α vivax malaria patients with anemia (97.83 ± 13.96 pg / ml). Red blood cells infected with Plasmodium falciparum sequestration experience in small blood vessels in organs. Sequestration was made possible by the expression of specific proteins on the surface of red blood cells that causes red blood cells infected can be attached to the adhesion molecules that arise at the endothelial surface of blood vessels as a receptor-ligand. Infected red blood cell adherence to the capillary and venule walls invite the other to blood cells clustered in those locations. At the time sizon in red blood cells are mature and experienced sequestration joint disintegration of red blood cells hospes, out of a number of antigens that stimulate monocytes and macrophages adjacent to produce TNF-α, IL-1, IL-6 and a number of other chemicals (Eling & Kremsner 1994). The outbreak of red blood cells infected sizon occur in the organ where the sequestration, the concentration of TNF-α in these places is very probably higher than in circulation, and biological effects can be felt already in place. If levels of TNF-α in the systemic circulation was found high as in this study, it is possible levels in the higher organs.

In falciparum malaria, there was sequestration of red blood cells infected in the bone marrow, also saw an increase in the number of macrophages between erythroblast, which phagocyte malaria pigment. This increased sequestration of TNF-α in bone marrow, causing anemia (Schlichterle et al. 1996). Plasmodium vivax does not have sequestration in small blood vessels in the organ, then sizon rupture can occur throughout the circulation so that the levels of TNF-α in the organ was not higher than in the general circulation.

Clark and Cowden (1999) states in Plasmodium vivax, cytokines have high levels in the circulation, but there is no local accumulation of cytokines in the organs. Mean levels of TNF-α in *P. falciparum* was significantly higher than *P. vivax*. This was caused by differences in the ability of TNF-α stimulate the production of Plasmodium species is the second antigen (Breitling et al. 2006).

Limitations of research

The first limitation of this study is limited research time, funds and facilities to conduct research. Second, the number of samples used in this studies only slightly, both groups of malaria patients with anemia or a group of malaria patients without anemia. Third, there is no baseline data on levels Plasmodium-infected patients prior to hemoglobin, causing difficulty in knowing the causes of decline in hemoglobin. Fourthly, no measurement of plasma levels of TNF-α prior to Plasmodium infection, these causes can not be known when the plasma levels of TNF-α began to show improvement. Fifth, Other diseases that accompany malaria (hepatitis, typhoid fever, urinary tract infection), only the patient anamnesis. Sixth, in this study can not be known how the mechanism of bone marrow suppression due to an increase in TNF-α. Based on some of the weaknesses that have been mentioned above, researchers suggested that further research be done by eliminating some of the weaknesses that have been mentioned.

CONCLUSIONS

From the overall results of this study can be concluded as follows: The mean plasma levels of TNF- □ in falciparum malaria patients with anemia are 145.27 ± 24.18 pg / ml, the mean plasma levels of TNF- □ falciparum malaria in patients without anemia is 16.18 ± 36.26 pg / ml, the mean plasma levels of TNF-□ on vivax malaria patients with anemia is 13.96 ± 97.83 pg / ml, the mean plasma levels of TNF- vivax malaria in patients without anemia is 14.57 ± 30.41 pg/ml, there are differences in plasma levels of TNF-□ on malaria patients with anemia and malaria without anemic patients, and showed that plasma levels of TNF-□ on malaria patients with anemia was significantly higher than plasma levels of TNF-□ anemic patients without malaria (p = 0.000 ...), increasing the number of Plasmodium in malaria patients will increase the amount of plasma levels of TNF-□ resulting in anemia.

Based on this research can be submitted several suggestions as follows: Conduct further research using more comprehensive data about the rate of hemoglobin before and after the patients infected with Plasmodium Plasmodium-infected patients, conduct further research regarding the ratio of IL-10 on TNF-□, because IL-10 inhibits TNF-□ the cause of anemia in patients infected with *P. falciparum*, conducted research on the mechanisms of bone marrow suppression in patients with anemia of malaria.

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