HEMATOLOGICAL CHANGES IN MALARIA

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HEMETOLOGICAL CHANGES IN *MALARIA* PATIENTS

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HEMATOLOGICAL CHANGES IN MALARIA

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ABSTRACT

The purpose of this study was to find the association of hematological changes and different types of malaria. In addition, the relationships between hematological changes in severe malaria, hemoglobinopathy and G6PD deficiency were investigated. A retrospective study was conducted of 204 confirmed malaria patients aged 12 years and above who had undergone antimalarial treatment.

The results showed that 56% of falciparum- and 61.9% of vivax-infected cases had decreased hemoglobin levels, with reduced WBC of 36.5 and 28.6%, in each group, respectively. However, thrombocytopenia was found in 88.7% of falciparum, and 90.5% of vivax, infections. Although the baseline hemoglobin and hematocrit levels of the uncomplicated and severe patients were lower than normal, they increased significantly over time, and approached normal by the end of the study. Total WBC counts were normal throughout the study period, with low neutrophil counts in the whole study, except for baseline. In addition, thrombocytopenia presented at baseline, returning to a higher level than normal after treatment but it became to normal in both patient groups at the end of the study. Hemoglobinopathy and G6PD deficiency did not appear significantly related to protectivity against malaria or severe malaria, with evidence of complications and history of malaria infection in all of these patients. In this study, all baseline hematological data levels were lower than normal in general, which may be due to hemolysis, hypoproliferative marrow from inflammatory cytokines and enhanced splenic destruction. However, most uncomplicated patients returned to normal within a short period, but it took longer for severe patients because of the aggressiveness of the disease.

KEY WORDS: MALARIA / ANEMIA / THROMBOCYTOPENIA / HEMOLYSIS

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LIST OF ABBREVIATIONS

Abbreviation or Symbol	Term
CBC	complete blood count
°C	degree Celcius
cm.	centimeter
ECG	electrocardiogram
G_6PD	glucose 6 phosphate dehydrogenase
g/dl	gram per deciliter
gm	gram
Hb	Hemoglobin
Hct	Hematocrit
Hr	Hour
IV	Intravenous
Im	Intramuscular
kg	kilogram
L	litre
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration

LIST OF ABBREVIATIONS (Cont.)

Abbreviation or Symbol	Term
mEq	miliequivalent
min	minute
MP	malaria parasite
pg	picogram
P.falciparum	*Plasmodium falciparum*
P.vivax	*Plasmodium vivax*
P.malariae	*Plasmodium malariae*
P.ovale	*Plasmodium ovale*
PTA	prior to admission
RBC	red blood cell
RDW	red cell distribution width
RR	respiratory rate
SC	subcutaneous
U	unit
WBC	white blood cell

CHAPTER I

INTRODUCTION

Malaria is the most important parasitic disease of man by looking from both clinical and public health perspectives. Now, people all over the world are being threatened by malaria especially those in tropical regions including Africa, South of the Sahara, South and South-East Asia, and parts of the America. Approximately, 5% of the world population is infected and estimated one million deaths each year (White NJ, 2003). In many of these regions, the burden especially socioeconomic burden has been increasing in alarming rate in recent years.

Malaria is protozoa infection which is born by Anopheles mosquitoes. There are nearly 500 species in the genus Anopheles, around 70 are capable of transmitting malaria, through with greatly varying efficiency (Krzywinski J et al., 2003) (Phillips RS, 2001). The Anopheles mosquitoes (female) transmit the disease by biting to human and with the saliva of the mosquitoes, the parasite enters into the human body via blood stream and proceed the disease process. Furthermore, it can be transmitted by direct inoculation of infected blood by means of congenitally, using of contaminated needle and blood transfusion.

There are four species of *Plasmodium* which are responsible for human malaria. The most severe and complicated one is *Plasmodium falciparum* which can give rise to malignant tertian malaria and answerable for most of the deaths every year. The other species are *P.vivax*, *P.ovale* and *P.malariae*, which can cause benign malaria.

In Africa, the most predominants one is *P.falciparum* malaria where as *P.vivax* is more common in Central and parts of South America, North Africa, the Middle East and the Indian Subcontinent *P. ovale* is relatively uncommon outside Africa as well as *P.malariae* is less common outside Africa.

Regarding disease process, some encounter only uncomplicated malaria although numerous people facing the severe complicated malaria which can lead to fatal. The common presentation of uncomplicated *P.falciparum* is fever, headache, fatigue, malaise, aches and pain, sometimes abdominal pain and diarrhea. For complicated one, addition to all presentations of uncomplicated malaria, there may be loss of consciousness, multiple convulsions, abnormal bleeding, circulatory collapse, pulmonary edema, respiratory distress all lead to death. Among these presentations, hematologic changes are one of the most interesting changes but specific changes may vary with level of malaria endemicity, background hemoglobinopathy, glucose 6-phosphate dehydrogenase deficiency and malaria immunity. Although many clinical trials are attempting to reach the development of new treatments and vaccines, the pathophysiology of the disease is incompletely understood in some points. Moreover, multidrugs resistant add up the tragedy to become more miserable, and hence it can delay researchers to achieve their goals.

CHAPTER II

OBJECTIVES

Primary Objective

To find the association of hematological changes in different types of malaria.

Secondary Objective

To observe the relationship between hematological changes in severe malaria, hemoglobinopathies and G6PD deficiency.

CHAPTER III

LITERATURE REVIEW

Malaria is a basic health problem especially in the tropical countries and it is responsible for thousands of deaths yearly. Malaria is found many parts of the world but endemic regions are Southeast Asia, Latin America, and sub-Saharan Africa (Krogstad DJ, 2001). The incidence of malaria in 2002 is 47,948 totally. According to Ministry of Public Health, Thailand, 21785 of *P.falciparum* infection, 25916 cases of *P.vivax,* 49 cases of *P.malariae* and 198 of mixed infection. Between 2000 and 2001, death rate rose from 201 to 429 cases in Thailand.

Malaria infection is due to malaria parasites and there are four species of parasites which are responsible for human malaria. It consists of *Plasmodium falciparum, Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. Out of four species of human malaria, *Plasmodium vivax* has the widest geographical range; it is prevalent in many temperate zones, but also in the subtropics and tropics. *Plasmodium falciparum* is the commonest species throughout the subtropics and tropics. *Plasmodium malariae* is patchily present over the same range as *Plasmodium falciparum*, but much less common. *Plasmodium ovale* is found chiefly in tropical Africa, but also occasionally in the West Pacific (SNOW RW *et al*., 2002).

Data from the WHO suggest that there are at least 200 to 300 million malaria cases and 1 to 2 million deaths each year (Krogstad DJ, 2001).

Incubation period of malaria is approximately two weeks but it can depend on the previous immunity, type of species and previously taking of ineffective antimalarial treatment or prophylaxis.The first symptoms of malaria are non-specific and all are similar for all four species of *Plasmodium*. High fever with shivering, mild chills which is associated with headache, muscular ache, vague abdominal discomfort, lethargy, lassitude, malaise and loss of appetite. Fever due to *P.vivax* and *P.ovale* infection is every 48 hours where as for *P.malariae*, it is every 72 hours. In contrast, in *P.falciparum* infection, fever and chills typically occur every 48 hours or it can be

without any periodicity. According to the life cycle of malaria parasites, red blood cells take part as critical site for production of asexual and sexual forms of *plasmodium* parasites. The merozoites from liver parenchyma cells undergo two forms of developments in red blood cells. In asexual stage, they develop from merozites to ring forms and gradually increased in size and nuclear division occurs to form matured schizonts. Not only asexual developments but sexual form also grows in red blood cells. In malaria infection, the parasitized RBCs becomes rigid and loss of deformability which lead to less flexible and more susceptible to damage in the circulation and more prone to splenic clearance. This mechanism does not mean only to parasitize RBCs, also to normal RBCs which pronounce more and more damage in blood (Warrell DA *et al*., 2002). The normal life-span of RBCs is 120 days. Moreover, the parasitized RBCs are destroyed by lysis in the blood stream because it acts as toxic substance in the erythrocytes environment. After intravascular hemolysis, RBCs release hemoglobin into the plasma and bound to plasma protein, heptoglobin and this complex is transported to the liver where they are metabolized into bilirubin and excreted to the intestinal tract via the bile duct. The normal hemoglobin concentration in plasma is less than 5mg/dl. However, in severe intravascular hemolysis, the amount of hepatoglobin in the circulation may not be sufficient to bind with hemoglobin and excess free hemoglobin accumulates in the plasma. At that time, another plasma protein, hemopexin, complexes with heme and this complex is cleared by the liver. If the level of free hemoglobin in the plasma exceed the amount that both of these mechanism can clear, they dissociate into dimmers which are small enough to pass through the glomerulus of the kidney and they may be reabsorbed by proximal renal tubules. However, if the rate of filtration exceeds that of reabsorption, free hemoglobin appears in the urine, which is the sign of rapid and severe intravascular haemolysis. Besides this mechanism, some free Hb which do not excreted by the kidney is quickly oxidized to methemoglobin. Then, methaemoglobin dissociates into hemin which is the oxidized form of the heme and globin. Hemin may bind to hemopexin if it is available or to albumin, forming methemalbumin which cannot be excreted in the urine. Globin goes to the amino acid pool and be used in protein synthesis.

The intravascular hemolysis can be detected by laboratory findings of hemoglobinaemia, hemoglobinuria, hemosiderinuria, methemoglobinaemia, decreased heptoglobin and decreased hemopexin. In addition, increased serum lactic dehydrogenase which is released from erythrocyte during hemolysis and increased bilirubin level can indicate intravascular hemolysis.

The malaria parasites not only affect the RBCs but also give erythropoietic changes in bone marrow by obstruction of marrow sinusoids by parasitized RBCs and dyserythropoiesis is resulted. Even after clearing the parasites, maturation defects still present for at least 2-3 weeks. Dyspoietic thrombopoiesis is observed by increased number of large, abnormal-looking megakaryocytes in marrow picture (Warrell DA *et al*., 2002). In Bangkok some investigators studied the course of anemia after the treatment of acute falciparum malaria. In this study, 72 adult Thai patients were followed up for 4 weeks after starting effective therapy. At weekly observations, the erythropoietic response of each patient with anemia was categorized. At 4 weeks, 56% of the patients were still anemic. Anemia may therefore persist in about one in every two Thai patients for up to 28 days after beginning effective treatment for acute *Plasmodium falciparum* malaria, hypoproliferative erythropoiesis appearing to be the most common mechanism of this anaemia (Camacho L.H *et al*.,1998). One descriptive study in Colombia was carried out in 104 patients with *P.vivax* malaria to observe the clinical and laboratory finding in *P.vivax* malaria. Anaemia was observed in 34 males and 14 females. The platelet average was 269,000/mm^3. Thrombocytopenia was present in 8 adult patients. Thrombocytopenia is a common finding in *P.falciparum* and *P.vivax* infection. The white cell count was abnormal in 34% of the patients: 29% leucopenia and 5% leucocytosis; the average of leucocytes was of 6200/mm^3. Eosinopenia and eosinophilia were 30% and 19% respectively (Echeverri M *et al*., 2003). One study was done in 1993 for hematological and coagulation profile in acute falciparum malaria. The number of cases was 30 and normocytic, normochromic anemia was observed in 86.7% of cases with complications. Severe anemia (Hb<6gm) in 10% of cases and leucocytosis and leucopenia were observed in 13.3% and 6.6% of cases respectively. 90% of cases had thrombocytopenia, the lowest count was 26,000 per/mm^3 and 16.7% of cases had evidence of intravascular coagulation (Sharma SK *et al*., 1993). Anemia is not

common in patients with *P.vivax* infection although dyserythropoietic changes are present. However, in the cases of mixed infection with *P.falciporum* and *P.vivax*, the survival of erythrocytes was found to be reduced. Regarding white blood cells series, mild leucopenia is common in uncomplicated malaria whereas neutrophil leucocytosis is a singnificant finding in severe falciparum malaria which reflex bad prognosis. In addition, platelet is also important for disease progress. The study in India about thrombocytopenia in malaria in 1565 subjects showed that normal platelet count was noted in 21.6% cases. The mean platelet count in vivax malaria (n=973) was 115,390/µl with a range of 8000-573,000/microl. In falciparum malaria (n=590), the mean platelet count was 100,900/µl with a range of 2000-497,000/microl. Platelet count<20,000/µl was noted in only 1.5% cases in vivax malaria as against 8.5% cases of falciparum malaria (Jadhav UM *et al*., 2004). In falciparum and vivax malaria, thrombocytopenia is common due to reduction of survival rate, sequestration and enhanced splenic uptake (Warrell DA *et al*., 2002).

There was one recent study of haematologic and clinical indices of malaria in a semi-immune population in Western Thailand. The total participants were 979 patients and 414 were infected with *P.falciparum* and 646 were infected with *P.vivax*. For falciparum and vivax infection, the mean values of WBCs, RBCs, platelet counts and lymphocyte counts were significantly lower for infected groups than for the uninfected groups. WBC<5000/µL, RBC<4,000,000/µL, platelet count <150,000/µL with history of fever for 3 days can tell the persons with any malaria. In *P.falciparum* infection, platelet count was less than 100,000/µL. The trend of decreasing platelet count with increasing levels of parasitemia observed in falciparum infection. Platelet consumption in disseminated intravascular coagulation contributes to thrombocytopenia in complicated falciparum infection. A person from this study population infected with falciparum is almost 15 times more likely to have a low platelet count (<150,000/µL) than a malaria negative one. Low WBC, RBC, and platelet counts were also significant for persons with *P.malariae* (Erhart LM *et al.*, 2004). In severe form of malaria, anemia is common with neutrophil leucocytosis and presence of visible malaria pigment in more than 5 per cent of circulating neutrophil is associated with bad prognosis. However, thrombocytopenia does not correlate with severity of disease unless it is profound (< 20,000 / µl). Prolonged prothrombin and

partial thromboplastin time and other evidence of disseminated intravascular coagulation are found in less than 10 per cent of patients. Plasma total and indirect bilirubin concentrations are increased consistent with hemolysis (Warrell DA, 2002).

The red cells polymorphism plays an important role in hematological changes of malaria. The impairment of globin chain synthesis in structure and the rate, the level of red cell enzyme (Glucose 6-Phosphate Dehydrogenase) are common factors in affecting the outcome of hematological changes and disease severity. In most cases, there is a strong evidence of protective activity of malaria in β thalassaemia and G_6PD deficiency (Marsh K, 2002). There are mainly two types of thalassaemia, α and β. In β thalassaemia, there is reduced rate of production of one or more of the β-globin gene synthesis and being common in Mediterranean basin, the Middle East and South-east Asia (Marsh K, 2002). Heterozygous HbE and β-thalassemia causes a moderate to severe thalassemia-like anemia. It is common in South-east Asians and the most severe type reveals only HbE and HbF where the amount of HbE is less than that of in homozygous HbE and the HbF is increased proportionately. HbE may also be found in combination with α-thalassemia which produces a more severe anaemia than HbE alone. Hb E is the commonest hemoglobin variant in the world. It is due to a single mutation of glutamine to lysine at position 26 in the β-chain. It is mostly common in South-east Asia. The protective mechanisms to malaria may be due to reduction of parasite growth in cells by exacerbation under conditions of oxidant stress and phagocytosis of parasitized red blood cells due to interaction of host immune system. It occurs in both parasitized homozygous and heterozygous cells (Marsh K, 2002). Homozygous HbE is characterized by the presence of a mild, asymptomatic, microcytic anemia with decreased erythrocyte survival. Target cells are prominent with decreased osmotic fragility with HbA_2 and HbF. HbE trait is symptomless and all hematologic parameters are normal except for slight microcytosis. The study in Bangkok, 2002 for hemoglobin E: a balanced polymorphism protective against high parasitemia and thus severe falciparum malaria in Thailand. The total subjects were 109 patients. Heterozygous hemoglobin E is functionally and relatively normal although microcytic and less Hb compared to other Hb but invasion of parasite is less than in homozygous. The study was carried out under standardized conditions ex vivo. *P.falciparum* preferentially invaded normal Hb

compared to abnormal Hb red cells. It suggested that Hb AE erythrocytes have an unidentified membrane abnormality that renders the majority of the RBC population relatively resistant to invasion by *P.falciparum*. Hence, it would not protect from uncomplicated malaria infection but would prevent the development of heavy parasite burdens (Chotivanich K *et al*., 2002).

The mechanism of protection to malaria is not quite certain but it may be due to thalassemic subjects show enhanced antigen expression on infected red cell surface and leading to facilitate immune clearance. In addition, parasite growth is reduced in β-thalassemic cells especially when exposed to oxidant stress (Marsh K, 2002) .

Human have four α-globin genes, one pair from each parent. In α-thalassaemia, defect in one or more of these genes and effects varying from one α gene affected (asymptomatic) to the absence of all four functional genes and in that condition, it is incompatible with life and results in stillbirth with Hb Barts hydrops syndrome. α-thalassaemia genes are found high frequency in parts of South-east Asia and in Melanesia (Marsh K, 2002). The study of influence on Hb levels in α thlassemia and microcytosis in *P.falciparum* infection in 494 nonhospitalized Nigerian children were observed. In contrast to non-thalassemic and heterozygous person, infection in homozygous patient did not influence Hb values. Microcytosis was significantly associated with protection from *P.falciparum* compared with normocytic anemic patient (Mockenhaupt FP *et al*., 1999). Another case control study of 301 Ghanaian children for α^+ -thalassemia protects from severe malaria with 2107 controls. It revealed that heterozygous α^+ -thalassemia reduced the odds of severe malaria by 48% (Mockenhaupt FP *et al*., 2004). Haemoglobin H disease is the disorder produced when three in four α.genes are deleted. It occurs most frequently in South-east Asia. The decreased syntheris of α-chanins creates a relative excess of β-chains, which unite to form tetreds of four β-chains. The hemoglobin produced from these β-tetrads is Hb H. In Hb H disease, the patient has some HbA mixed with HbH and has high oxygen affinity and causes chronic hemolytic anemia due to unstable precipitating. Hb H is particularly sensitive to oxidation, forming intracellular precipitates in older cells where there is an increase in methemoglobin formation. The erythrocyte lifespan is shortened as these inclusions injure the cell membrane and lead to cell sequestration in the spleen and phagocytosis by mononuclear phagocytes. G6PD is the first enzyme

of the hexose monophosphate pathway and it is the main role of NADPH production. The gene for G6PD is on the X-chromosome and male hemizygotes show severe deficiency but not common in female homozygotes. It occurs most frequently in the Mediteraanean area, Africa and China. The majority of people with G6PD deficiency have no clinical expressions of the deficiency unless they have neonatal jaundice, are exposed to chemicals or drug oxidants, or have severe infection (Marsh K, 2002).When malaria parasite use host pathway for NADPH production, it encounter the changes in the level of the enzymes. Hence, parasite growth is inhibited in G6PD deficient cells.

The treatment of malaria depends on the infecting plasmodia species, the geographic area of drug resistance and the severity of infection. Falciparum malaria in the nonimmune person is a medical emergency and requires rapid initiation of antimalarial therapy. If the species cannot be identified immediately, the patient should be assumed to have drug-resistant falciparum malaria until proven otherwise. Uncomplicated falciparum malaria may be treated with oral therapy whereas for severe type, it needs parenteral therapy (Maguire JD *et al*., 2002).

Artemisinin (Qinghaosu) is an endoperoxide- containing compound extracted from the leaves of the plant Artemisia annua (sweet wormwood). It is isolated by Chinese scientists in 1972. Because of high activity and low resistance make it to play a critical role in antimalarial treatment. Artemisinin is a sesquiterpene lactone, containing a labile peroxide bridge. The breakdown of this bridge generates free radicles which rapidly undergo alkalating reactions. Parasite membrane is particularly sensitive to this oxidative damage. Haemozoin catalyze the decomposition of the drug. This haemozoin is critical in particularly sensitivity of malaria parasite artemisinin action in to malaria. Lacking of haemozoin can resist to artemisinin. Artemisinins are used parenterally and by suppository for treating severe multiresistant falciparum malaria. Artemether is available for intramuscular injection as well as artesunate, it has water soluble property and it is haemisuccinate ester, can be administered intravenously or intramuscularly in solution as artesunic acid, or orally as 600mg. Artemisinin and artemether suppositories are available commercially. Adverse effects are very rare except neurotoxicities in animals with very high dose. Because of its very rapid action effect and less side, there is great

interest to use it in combining with other antimalarial drugs which can give the sense of expectancy to reduce the distressing drug resistant malaria in the future.

Mefloquine forms complexes with haemin and block haemin release with a protease inhibitor antagonizes its antimalarial activity. Mefloquine works by interaction with haemin and its polymer. It can against the asexual stages of all species of human malaria parasites. It has no power to kill gametocytes and no useful activity against the exo-erythrocytic stages of *P.vivax* or *P.ovale*. Its resistance is common in South-east Asia. Oral bioavailability is about 80% and it is highly lipid-soluble and extensively distributed especially in lungs. Plasma protein binding capacity is more than 95%. Unchanged drugs and metabolites are eliminated mainly via the bile in the faeces. Half-life is very long, ranging from 15 to 33 days. It is widely used for the prophylaxis and treatment of uncomplicated multiresistant falciparum malaria. Its combination with artesunate proved effective against uncomplicated falciparum malaria in areas where high level of resistance to mefloquine alone. The most common adverse effects are unpleasant gastrointestinal symptoms such as nausea, vomiting, coliky abdominal pain and diarrhoea. Other rare and serious side effects are exfoliative dermatitis, toxic epidermal necrolysis, cutaeneous vasculitis and aplastic anaemia. The use of mefloquine during pregnancy increases the risk of stillbirth and so it is contraindicated to pregnancy (Warrell DA *et a*l., 2002).

Despite the extensive spread of chloroquine resistant strains of P.falciparum and P.vivax infection, it is still the most widely used antimalarial drug in the world. Chloroquine remains effective for *P.vivax*, *P.ovale* and *P.malariae* infections worldwide and for falciparum malaria in restricted areas such as Central America, northwest of the Panama Canal, Haiti and the Dominican Republic and parts of Middle East. It is a rapidly effective blood schizonticide and also has the action of gametocide against *P.vivax*, *P.malariae* and *P.ovale*. In mode of action of Chloroquine, parasite's food vacuole is its target. In addition, it interferes the detoxifying mechanism of parasites to ferriprotoporphyrin IX and superoxide anions that resulting from its digestion of haemoglobin and hence the parasite is killed by accumulation of these toxic products. In healthy adults and patients with uncomplicated malaria, ingested chloroquine is rapidly absorbed. After intramuscular

or subcutaneous injection, absorption is very rapid and dangerously high peak plasma concentrations (500-3500 µg/L) may be reached within 5-20 minutes after a dose of 5mg base /kg. It can be prevented by giving small, more frequent injection. About half of the absorbed chloroquine is cleared unchanged by the kidney. The rest is biotransformed in the liver.

Chloroquine is used for the treatment and prophylaxis of vivax, ovale and malariae malarias and for uncomplicated chloroquine-sensitive falciparum malaria. If the plasma chloroquine level exceeds 250µg/ml, adverse effects such as dizziness, headache, diplopia, disturbed visual accommodation, dysphagia, nausea and malaise may develop especially after intravenous infusion. Systolic hypotension and ECG abnormalities may occur during the initial distribution phase. Rare toxic effects are photoallergic dermatitis, aggravation of psoriasis, skin pigmentation, leucopenia, bleaching of the hairs and aplastic anaemia. Chloroquine can exacerbate epilepsy.

Overdose of chloroquine can give acute or chronic toxicity. The acute toxicity produce symptoms within 30 minutes to 6 hours include nausea, headache, drowsiness, blurring of vision, malaise, vomiting, speech abnormalities, jaw contractions, hypokalaemia, thrombocytopenia, coma, convulsions, hypotension, respiratory paralysis and cardiac arrest. ECG changes include sinus tachycardia, bradycardia, prolonged QT interval, ectopic beats, ventricular tachycardia and fibrillation, idioventricular rhythm and asystole. If chloroquine is taken continuous weekly for antimalarial prophylaxis, it can be lead to chronic toxicity (Warrell DA *et al*., 2002).

Primaquine, and 8-aminoquinolilne is the drug of choice for the radical cure of P.vivax and P.ovale infection. Primaquine is active against exo-erythrocytic schizonts and gametocytocidal for all species of human malaria parasite and hypnozoitocidal for *P.vivax* and *P.ovale*. Primaquine is rapidly metabolized in liver and half-life is 5-6 hours. Primaquine is used for the redical cure of vivax and ovale malarias and also gametocytocidal drug against *P.falciparum* to a very limited extent. The adverse effect is acute intravascular haemolysis and methaemoglobinaemia in G6PD deficiency patients which is most severe in people with Mediterranean and Asian variants of G6PD deficiency. Gastrointestinal disturbances are also described (Warrell DA et al., 2002).

CHAPTER IV

MATERIAL AND METHODS

3.1 Study Site, Study Design and Study Period

This study will be done in Hospital for Tropical Disease, Bangkok. It is the retrospective study of hematological changes in malaria in the patients who were admitted to that hospital from 2003 to 2004. The study period is from November, 2005 to January, 2006.

3.2 Ethical Concern

Patients included were part of larger compatible clinical trial in process at the Hospital for Tropical Disease. All these clinical trials have been approved by the Ethical Committee and the signature of an informed consent form is prerequisite for inclusion into these clinical trials.

3.3 Inclusion Criteria

1. The patients who had malaria infections and confirmed by thick and thin blood film microscopically were admitted to the Hospital for Tropical Disease, Bangkok from 2003 to 2004.
2. The patients who were explained about the trial including all consequences of the trial and signed the written informed consent.
3. All the participants must be older than 12 years old and both genders can be accepted.
4. All the patients who took part in the study could be feasible for 28 days follow up.
5. All the patients must have hospitalization at least 7 days.
6. All the patients received according to their species of plasmodium infection. *P.falciparum* infected patients were given Artesunate 600mg/day for 3-5 days

plus Mefloquine 1250mg/day single dose on day -2. For vivax and ovale infections, patients were given chloroquine 1 g (600mg base), then 500mg (300mg base) 6 hr later, then 500mg (300mg base) at 24 and 48 hr plus primaquine 15mg base/day for 14 days . For malariae infection, the same dose of chloroquine in treatment of vivax and ovale infection is used.

3.4 Exclusion Criteria

1. Pregnant women and lactating mother are not suitable for this study.
2. Persons who have known hypersensitivity to prescribed drugs.
3. Presence of other concurrent illnesses is not considered for the trial except worm infestation which contributed up to 70%.
4. Presence of mixed infection at the admission can not include in the study.

3.5 Sample Size

Estimately and retrospectively, 204 patients were included in the study and it is the available amount that can get within two years of study from 2003 to 2004.

3.6 Methodology

1. All demographical details, the physical examinations, hematological, parasitological, biochemical tests were carried out in all participants of the trial which are followed the routine procedures at the Hospital for Tropical Diseases, Bangkok.
2. The sample size is estimately 204 patients and blood was taken from every patient on Day 0 and thick and thin blood films were done to differentiate the parasites species and detect the parasites count. In addition, all hematological examinations such as Hb level, Hematocrit (HCT), MCV, MCH or MCHC, total WBC count, differential count, RDW and platelet counts were done. Moreover, all hemoglobin typing and G6PD assay were done by using hemoglobin electrophoresis and fluorescent spot test respectively. In addition, biochemical tests such as blood glucose, urea and creatinine, total and differential bilirubin level are measured.

All the investigation procedures were determined at baseline and follow up period of day 7, 14, 21, 28 and the results were recorded.

3. All the patients were treated according to their species of plasmodium infection. *P.falciparum* infected patients were given Artesunate 600mg/day for 3-5 days plus Mefloquine 1250mg/day single dose on day -2. For vivax and ovale infections, patients were given chloroquine 1 g (600mg base), then 500mg (300mg base) 6 hr later, then 500mg (300mg base) at 24 and 48 hr plus primaquine 15mg base/day for 14 days. For malariae infection, the same dose of chloroquine is used in treatment of vivax and ovale infection.

4. The outcomes of the study is assessed by routine physical examination and observing vital signs such as body temperature, respiratory rate, blood pressure and pulse rate everyday until discharge. Laboratory investigations of hematological parameters like rbc, Hb level, hematocrit (Hct), MCH or MCHC, RDW, mean Corpascular Volume (MCV), total WBC, differential count of WBC eg, Neutrophil count, Lymphocyte, monocyte, Basophil, Eosinophil count and platelets. Parasite clearance time (PCT), (the time from the start of treatment until the first negative blood film and remained negative for the next 24 hours) and fever clearance time (FCT), (the period from the start of treatment until the oral temperature decreased to 37°C and remained below this temperature for the next 48 hours) also included in the parameters. Fever is measured four hourly and parasite count is assessed six hourly during parasitemia until it becomes negative. After that, thick blood film was done everyday until discharge. Density of parasitaemia by counting in a thin blood film is the number of asexual parasites per microlitre equal to the number of asexual parasites per 1000RBC into total RBC in 10^6/µl and for the thick blood smear, it is equal to the number of asexual parasites per 200 WBC into total WBC in 10^3/µl

3.7 Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Science. All the *P-values* reported were from 2-tailed tests and the statistical significance level was set at 0.05. The distribution of data was assessed for normality using the (Kolmogorov-Smirnov test or Shapiro-Wilk test). Data were expressed as

means and SD. The Statistical tests will be performed by using these methods appropriately: (1) chi-square analyses to test differences between 2 groups of the qualitative variables, (2) independent t-test to test differences between 2 groups of quantitative variables in demographics and baseline laboratory data,(3) repeated measure ANOVA to test differences in each item of the laboratory data at each follow-up, with respect to data baseline levels, (4) linear regression to explain variation in values of one variable by other variables and to test linear relationship between variables and (5). The Pearson's correlation is used to find a correlation between at least two continuous variables. However, in case of data do not generally show normal distribution. The data will be shown as median and range. Statistical test will be performed as appropriate 1)Chi square to test the differences among qualitative variables, 2) Mann-Whiteney *U* test for the differences between quantitative variables and 3)Kruakal Willis test to test differences among quantitative variables.

3.8 Significance of the Research

(a). Demonstrate hematological changes after treatment

(b). Find out association (Hb-typing, G6PD deficiency) with malaria infection.

3.9 Research Fund

The research fund has been provided by the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

CHAPTER IV

RESULTS

A total of 204 patients fulfilled the inclusion criteria for this study. There were 106 patients were comprised to uncomplicated group and 98 patients were in severe group. Age fluctuated between 13 and 49 years old with median value 24 for uncomplicated malaria group and that of for severe group, it was 21 years old with a range of 13-53, (p=0.671) . There was a male predominance in both group, male and female ratio was 2:1 for uncomplicated group where as 1.5:1 for severe group. Regarding the body weight and height of the patients, there was no significant relation to severity of malaria (p =0.302 and p =0.287 respectively). When malaria is observed by species, the total number of patients with *Plasmodium falciparum* infection was 159 (77.93%), patients with vivax infection was 42 (20.6%), that of with malaria infection was 2 (0.98%), and ovalae infection was 1 (0.49%).When we observed the malaria species with ethnic group, 57 Mon patients (35.8%), 44 Karen (27.7%), 27 Myanmar (17%) and 31 Thai (19.5%) were infected with *Plasmodium falciparum* and 10 Mon (23.8%), 15 Karen (35.7%), 11 Myanmar (26.2%) and 6 Thai (14.3%) were infected with *Plasmodium vivax*. There were two *Plasmodium malariae* patients, one Myanmar and one Mon patient. There was only one *Plasmodium ovale* infection and it was seen in Myanmar patient. Regarding the uncomplicated and severe malaria infection group, Mon patients comprised 34 (32-34%) for each group and Karen people (23% for uncomplicated and 34% for severe group) became second most out of four ethnic groups. For Myanmar (19% for each group) and Thai (24% for

uncomplicated and 11% for severe group) patients, it is not quite different in number. When we studied the residence province with different types of malaria, 54.1% of falciparum infection, 64.3% of vivax infection, 1 malariae infected patient and one ovale patient were from Tak province. Moreover, 37.1% of falciparum infection, 28.6% of vivax infection and one malariae infection were associated with Kanchanaburi province. From Ratchaburi province, only 8.8% of falciparum infection and 7.1% of vivax infection were found. When severity of malaria was related to residence province, most of the patients from both groups stayed in Tak Province (57 patients for uncomplicated and 58 for severe malaria group) and it contributed to 56%. People from Kanchanaburi province are the second most common (33 for uncomplicated and 39 for severe group) which was 35%. The remaining were from Ratchaburi Province (16 for uncomplicated and 1 for severe group) and it comprised of 19%. When we observed each place by comparing uncomplicated and severe group, there was no significant difference in patients from Tak and Kachanaburi whereas there was statistically significant in Ratchaburi group where 16 patients in uncomplicated group and one patient in severe group ($p = 0.001$).

Most of the patients (80%) were laborer and others were farmers and monks. There was no significant relationship between the occupation of patient and severity of the disease ($p = 0.634$). Furthermore, smoking and alcohol drinking were independent form severity of the disease ($p = 0.33$ and $p = 0.102$ respectively). After that, previous experience of malaria was observed and the result came out. Out of different species of malaria, 27.7% of falciparum infected patients and 40.5% of vivax patients had history of previous experience about malaria. It showed that 43 uncomplicated patients (40.6%) had experience but only 18 patients (18.4%) from severe group had previous attack history. When baseline investigations were studied, except body temperature, the remaining such as respiratory rate, pulse rate, blood pressure (both systolic and diastolic) were significant ($p<0.001$) Table (1).

Out of 159 patients with falciparum infection, 79 patients had liver enlargement (49.7%), 10 patients of vivax infection (23.8 %) had enlarged liver but there was no liver enlargement in malariae and ovale infection. However, only 11 patients (6.9%) from falciparum infected group, 1 patient (2.4%) from vivax group had enlarged spleen.

After doing thick and thin blood film for diagnosis of malaria infection, the median value of initial parasite count for uncomplicated group was 8520 (1-245960) and that was apparently lower than that of for severe malaria group which was 170640 (60-1277500) ($p<0.001$). In addition to this, the parasite clearance time for uncomplicated patients was less than that of for severe patients, 42 (18-331) and 48 (9-96) respectively and there was correlation between uncomplicated patients and severe patients by means of parasite clearance time ($p<0.001$). Moreover, fever clearance time was related to severity of the disease which meant that the patients without complication had shorter fever clearance time (Median = 24, Range=4-160) than the patients with complications (Median= 68, Range = 4-232) ($p<0.001$) Table (2). When the complications of malaria were studied, it was found that 12(11.3%) uncomplicated patients had anemia clinically while 60(61.2%) severe patients had the same effect and it was statistically significant ($p=0.026$). Furthermore, to know the frequency of complication of malaria in each malaria infection, we used crosstab method to analyze it. Concerning cerebral malaria, only 10 patients from falcipaum infection (6.29%) got cerebral malaria whereas other species didn't cause it. Moreover, 5 falciparum infected patients were treated by dialysis for acute renal failure which was (3.14%). In addition to this, hyperparasitemia was found only in 57 falciparum infected patient (35.85%) and the hemoglobin level of 11 falciparum infection and 1 vivax infected patient was less than 7 mg/dl. All of the complications in each species of malaria was insignificant statistically ($P>0.05$).

After that, there was more analysis to know that whether there was relationship of anemia and other parasitic infection or the anemia was due to the effect of malaria only. The frequency of worm infestation in both groups was 70%. There were only 9(12%) uncomplicated patients who had both anemia clinically and parasitic infection but for severe group, it was 45(64%) anemic patients with other parasitic infestation. In other words, there were only 9 patients from uncomplicated group out of totally 74 patients with other parasitic infection but 45 patients out of 70 with other parasitic infection had anemia in severe group. When the baseline hemoglobin level was related to sex, female patients were more anemic than male patients and 61 female (80%) patients in 77 total female had hemoglobin lower than normal value and 56 male

patients (44.06%) out of 127 had low hemoglobin than normal and all were statistically significant (p<0.001).

The anemic patients were treated by giving packed red cell, whole blood or fresh frozen plasma. Only one (0.9%) uncomplicated patient and 38(38.8%) severe patients received packed red cell transfusion. For whole blood, only 4 severe patients got it and it was 4.1%. Furthermore, there were 46 complicated patients (46.9%) were transfused fresh frozen plasma. When jaundice was taken account into analysis clinically, only 8 (7.5%) uncomplicated patients had jaundice but 53 (54.1%) of severe patients encountered jaundice. Then, the relationship between anemia and jaundice was studied clinically. 20 patients had jaundice but no anemia while 41 patients had both jaundice and anemia. 31 patients had anemia without jaundice and this relationship was statistically significant (p<0.001). Again, one of the factors which link to severity of malaria was parasite density and out of 98 severe patients, 57 patients (58%) faced with hyperparasitemia. Moreover, acute renal failure and hemoglobinurea also contributed to certain degree in accessing of disease severity. There were 10 severe patients got acute renal failure and 5 of them (50%) were treated by hemodialysis which was significant statistically (p<0.001) but one patient from severe group suffered from hemoglobinurea without having renal failure (p=0.735). When cerebral malaria was related to other complications, only 6 severe patients (6%) faced with both hyperparasitemia and cerebral malaria. Other 50 patients (51%) had hyperparsitemia but without cerebral malaria. However, 4 patients (4.1%) with cerebral malaria did not get hyperparasitemia. The relationship between cerebral malaria and hyperparasitemia was not statistically significant (p=0.687). Besides this, there was no correlation between cerebral malaria and severe anemia (Hb < 5 mg/dl – WHO recommendation) (p= 0.972) and there was no patient with cerebral malaria together with severe anemia. Nevertheless, out of 10 patients with cerebral malaria, 3 patients suffered from acute renal failure and treated with hemodialysis. In addition, out of 5 patients who treated by haemodialysis, 3 patients got cerebral malaria which was statistically significant (p=0.001).

When hemoglobin electrophoresis and quantification had been undergone, patients with normal hemoglobin were 49 for uncomplicated group and that was for severe group was 47 (p=0.804), there were 27 patients from uncomplicated group and

15 patients from severe group were beta-trait (p=0.073), 5 from uncomplicated group were haemoglobin-E (homo) (p=0.029), 8 patients from uncomplicated group and 10 patients from severe group were Hb E – trait (p=0.504), one patient from severe group was Hb-H (p=0.297),one patient from uncomplicated group had alpha trait (p=0.335), 6 people from uncomplicated group and 12 from severe had beta homozygous (p=0.098), 10 uncomplicated patients and 13 severe patients were supposed to have iron deficiency anemia (p=0.387) but it was not sure as we could not do investigations to confirm it so put it in unidentified group. When we observed the relationship between red cell enzyme deficiency and degree of severity of the disease, there was no link to each other (p=0.248). It was found that not only in patients with normal hemoglobin but also in patients with hemoglobinopathies and G6PD deficiency, there was presence of previous history of malaria before. Furthermore, patients of hemoglobin E homozygous , patients with beta thalassemia homozygous were less common for hepatosplenomegaly and presence of complications of malaria compared to that of patients with heterozygous pathology. Alpha trait patients had previous history of malaria without hepatosplenomegaly and complication. Hemoglobin H disease patients and G6PD deficient patients had both previous history of malaria and hepatosplenomegaly and some complications. The most surprising finding was that there was no patient with severe anemia in hemoglobinopathies and G6PD deficient patients whereas severe anemia was found in normal hemoglobin patients Table(3).

Hematological Results

When we observed the hematological changes in different malaria, on baseline, 56% of falciparum infection and 61.9% of vivax infected patients had decreased hemoglobin level lower than normal (11.6 and 11.5 mg/dl respectively). One week after treatment, it reduced more than the baseline in both infections (10.4 and 11.15 mg/dl respectively) and then it went up day by day and at the end, it became 12.1 mg/dl for each infection Figure(6). In total leucocytes series, the baseline was just above the lower normal for both species (5.7×10^9/L for falciparum infection and 5.55×10^9/L for vivax infection) and it became increased the level in a few days and at the end of the study, it became 8.2×10^9/L for falciparum infection and 7.3×10^9/L for vivax infection Figure(7). The baseline platelet level were very low for both species

(50.5×10^9/L for falciparum infection and 79×10^9/L for vivax infection). Immediately after taking treatment, it became shoot up to the level of thrombocytosis (313.5×10^9/L for falciparum infection and 406.5×10^9/L for vivax infection which was higher than in falciparum). Then, it showed the decreasing trend continuously to maintain the normal level in the following weeks and at the end of the study, it became 241×10^9/L for falciparum infection and 214.5×10^9/L for vivax infection Figure(8) Figure(5).

In addition, hematological profiles of malaria infected persons without complication were compared with those of persons with complication. It was not only account for the first day but also for the consecutive weeks during the study period. The median hemoglobin value 116 mg/dl for Day 0 was distributed evenly between the uncomplicated and severe malaria group but the range for both groups is different, 67-165 and 53-171 respectively. The changes of hemoglobin level affected equally to both groups and there was no specific significance (p=0.375). When we observed hemoglobin value for subsequent weeks, there was some significance in some points. The level of hemoglobin in both types of the patients was decreased in small amounts in Day 7 compared to Day 0. However, the hemoglobin level of both groups rise up to a certain level in the following days which started from Day 14 up to Day 28. In that case, both groups were compared and it was found that the differences were apparent Fig (2).

Secondly, hematocrit level of all patients was studied. The baseline result were 35 % for median value of uncomplicated patients (21-51 %) and 34 % for that of severe patients (17-49 %) and there was significantly different in both groups (p=0.029). The median haematocrit level of both groups were analyzed up to Day 28, the degree of the disease and respond to treatment was highly depend on the level of haematocrit and it was significant for Day 0,7,14,21 and 28 Fig(1). Generally, hemoglobin and hematocrit level of uncomplicated and severe patients were below normal on baseline but after treatment, it increased gradually up to nearly normal at the end of the study. In that case, it was found that the respond of uncomplicated patients were better and faster than that of severe group to touch normal.

When white blood cells were analyzed, the median value of total WBC count on Day 0 was 5.7 ($x10^3$ /µl) (2.6-11.6) for patients with no complication and it was 5.8 (1.6-25) for patients with complication and there was no vivid difference. When it was

studied continuously, it increased to a certain amount on Day 7 but it went down on Day 14 when there was quite significant difference. On Day 21, the level of both groups were nearly overlapped while on Day 28, there was big difference which was due to rising up of the line of severe group which was almost always below the level of uncomplicated group before. All the changes in total WBC were within normal limit in the whole study Fig(3) Table(4).

With regards to WBC differential, on the first day, all were apparently different for both groups except basophil which was the same for both groups with a few different in range. The study of differential counts proceed after beginning effective treatment for malaria according to whether the patient had uncomplicated or severe disease. Generally, there was certain significant difference of segment, eosinophil and lymphocytes levels until first two weeks. Although the significance in eosinophil and segment level stopped after two weeks, that of lymphocytes were continued up to third week. The graph of segment showed difference in second week though it did not occur in third week. The level of segment was nearly upper normal on baseline but it decreased for both groups in the following weeks and reached to below normal until the end of the study. Lymphocytes, monocytes and eosinophil level were higher than lower normal on baseline but high up within few days after the treatment. In the case of eosinophil, it reached to more than normal on the last day of study. Basophil level was within normal and did not show much changes in this study. In case of band form, it was higher in severe patients on baseline and after the treatment, it became negative for both types of patients. The significant difference of monocytes showed only on Day 0 to Day 7 and later it resolved. The basophil level showed significance only in Day 14 and Day 21, Fig (5-9). The platelet level on baseline was 98x 10^3/ µl (26-309) and 30x10^3/µl (5-457) for uncomplicated and severe group respectively. Thrombocytopenia was observed on baseline but became thrombocytosis within first week after the treatment. Then, it returned to normal slowly and stayed within normal at the end of the study in both groups. It still showed significance up to at the end of the first week and it appeared again on the last day of study period Fig (4).

Biochemical Findings

When we observed biochemistry, BUN, creatanine were important for renal function. The baseline data for median value of BUN was 13.1 mg/dl (5.2- 24.7) for uncomplicated malaria infection and 24.2 mg/dl (9.7-119.8) for severe group which was above normal and there were significant differences for both data ($p<0.001$ for each).Within one week after treatment, it went down to normal and both groups maintain to be within normal (11mg/dl for each group) until at the end of the study. During the study period after starting the effective treatment, there was no correlation between uncomplicated and severe malaria for BUN whereas creatanine level become significant on baseline and Day 21 but it was within normal in the whole study period (<1 mg/dl).

The baseline biochemical findings such as direct bilirubin, total bilirubin, AST, ALT, Na^+ and Cl^- showed difference in uncomplicated and severe groups significantly ($p<0.001$ for each group). Nevertheless, alkaline phosphatase level and potassium level were not significant properly ($p=0.151$ and 0.654 respectively) through out the study period. Both direct bilirubin and total bilirubin were significant throughout the study period Fig (13,14). Both uncomplicated and severe patients associated with high total bilirubin (1.5 mg/dl for uncomplicated and 3.5 mg/dl for severe patients) with high direct bilirubin only for severe patients (1.8 mg/dl for severe patients and 0.45 mg/dl for uncomplicated).However, uncomplicated patients had association with high indirect bilirubin level (1.05 mg/dl) and severe patients with very high level of indirect bilirubin level (1.7 mg/dl). After effective treatment, all the levels of bilirubin in both groups reached to normal limit within one week. Regarding ALT and AST, severe patients had high level on baseline (>50 U/L and >60 U/L respectively) but they were still normal in uncomplicated patients. ALT level of severe patients touched to upper normal only at the end of the study whereas AST level became normal two weeks after treatment. high ALT also showed significance from D0 up to D28 Fig (15) whereas AST gave it only in D0, D7, D14 and D21. Regarding the electrolyte, sodium level of severe patients were slightly below normal on baseline but after taking treatment, it returned to normal until at the end of the study and it was significant in D0, D7 and D28 Fig (17) while chloride showed the significance only in D0 and D 7.

To know the cause of anemia whether it was due to hemolysis or due to liver function impairment or due to combination of both mechanisms, we made the correlation of hemoglobin day 0 with indirect bilirubin day 0 and direct bilirubin day 0 by using crosstab. It showed that in both uncomplicated and severe group, there were more patients who had both lower hemoglobin level (< 5 mg/dl) than normal and high indirect bilirubin compared to direct bilirubin level. Relationship between hemoglobin day 0 and indirect bilirubin day 0 was not significant for both uncomplicated and severe groups whereas there was significance for severe malaria group in comparing hemoglobin day 0 and direct bilirubin day 0 ($p=0.041$). Indirect bilirubin showed significance in Day 0,7,14,21 and 28 ($p<0.001$ for all except Day 21 $p=0.044$ and Day 28 $p=0.16$). Moreover, the correlation between hemoglobin day 0 and albumin day 0 was significant for both uncomplicated and severe group ($p<0.001$ for each group). The baseline albumin level which was lower than normal was distributed equally in both sex but different percentage (41.7 % for male and 68.8 % for female).

For the study of biochemistry, 17 uncomplicated patients and 71 severe patients were found BUN level more than normal (>19mg/dl). Regarding creatanine, the number of patients who had high level of creatanine (> 1.4 mg/dl) were 2 patients from uncomplicated and 19 from severe group. The BUN level and creatanine level for both uncomplicated and severe group were significant ($p < 0.001$). When we compared the BUN and creatanine level with dialysis, 5 patients with high level of creatanine and 5 patients with high BUN level were treated by dialysis out of 21 patients had higher creatanine level and 88 patients had higher BUN level. Hence, it showed statistically significance ($p< 0.001$ for creatanine, $p = 0.009$ for BUN). When we studied the relationship between dialysis and BUN level > 60 mg/dl and creatanine > 3 mg/dl for severe malaria, there were 5 severe patients with BUN more than 60 and also 5 severe patients with creatanine more than 3 got hemodialysis ($p< 0.001$ for each one). The total number of patients with BUN level more than 60 was 11 patients and that of with creatanine level more than 3 were 6 patients. When we observed each patient, it was found that 5 patients with BUN more than 60 were equal to that of with creatanine more than 3. It meant that those 5 patients had both high BUN and creatanine level and they were treated by hemodialysis.

Table 1. Results of demographic data of uncomplicated and severe malaria patients in absolute and percentage value with their significance level.

	Uncomplicated Malaria	Severe Malaria	p-value
Sex			
Male	70 (66%)	57 (58%)	
Female	36 (34%)	41 (42%)	
Ethnic Group			
Mon	34 (32%)	34 (34%)	
Karen	25 (24%)	34 (34%0	0.064
Myanmar	21 (19%)	19 (20%)	
Thai	26 (25%)	11 (12%)	
Residence Province			
Tak	57 (53%)	58 (59%)	
Kanchanaburi	33 (32%)	39 (40%)	
Ratchaburi	16 (15%)	1 (1%)	0.001
Occupation			
Laborer	86 (81%)	84 (81%)	
Farmer	18 (17%)	12 (17%)	
Monk	2 (2%)	2 (2%)	
Previous History of Malaria	43 (40.6%)	18 (18.4%)	0.002

Table 2.Result of demographic data, clinical findings and laboratory data (baseline) in uncomplicated and severe malaria patients in median and range with significance level

	Uncomplicated Malaria	Severe Malaria	p-value
Age (years old)	24 (13-49)	21(13-53)	
Weight (kg)	41 (35-83)	50 (25.5-84.8)	
Height (cm)	161 (131-181)	160 (146-186)	
Fever before admission (days)	4 (1-30)	5 (2-14)	<0.001
Temperature (Ċ)	38.5 (36-40)	38.3 (36-41.9)	0.054
Respiratory Rate (per minute)	20 (20-34)	24 (18-40)	<0.001
Pulse Rate (per minute)	90 (72-120)	100 (72-208)	<0.001
Blood Pressure (in mmHg)			
Systolic	110 (90-130)	100 (67-143)	<0.001
Diastolic	70 (50-90)	60 (26-90)	<0.001
Initial Parasite Count	8520 (1-245960)	170640 (60-1277500)	<0.001
Parasite Clearance Time (hours)	42 (18-331)	48 (9-96)	<0.001
Fever Clearance Time (hours)	24 (4-160)	68 (4-232)	<0.001

Table 3. Number of patients with normal, abnormal hemoglobin and G6PD deficiency in uncomplicated and severe malaria patients.

	Uncomplicated Malaria	Severe Malaria	Total Number of Patients
Normal Hb	49	47	96
Beta (trait)	27	15	42
Beta (homozygous)	6	12	18
Hemoglobin E (trait)	8	10	18
Hemoglobin E (homozygous)	5	0	5
Hemoglobin H	0	1	1
Alpha (trait)	1	0	1
Unidentified (Iron Defiency Anemia ??)	10	13	23
G6PD deficiency	7	3	10

Table 4. Result of baseline hematological findings in uncomplicated and severe malaria patients in median and range with significance level.

	Uncomplicated Malaria	Severe Malaria	P-value
Hematology			
Total WBC ($\times 10^9$/L)	5.7(2.6-11.6)	5.8(1.6-25)	
Hemoglobin (mg/dl)	116(67-165)	116(53-171)	
Hematocrit (%)	0.35(0.21-0.51)	0.34(0.17-0.49)	0.029
Platelets ($\times 10^9$/L)	98(26-309)	30(5-457)	<0.001
Bandforms (%)	1(0-17)	3(0-30)	<0.001
Segment (%)	64(23-85)	67(29-88)	0.014
Eosinophils (%)	2(0-10)	1(0-17)	0.005
Basophils (%)	1(0-2)	1(0-3)	
Lymphocytes (%)	21(5-51)	19(5-49)	0.002
Monocytes (%)	9(2-13)	5(1-12)	<0.001

Table 5. Result of baseline biochemical findings in uncomplicated and severe malaria patients in median and range with significance level.

	Uncomplicated Malaria	Severe Malaria	p-value
BUN (mg/dl)	13.1 (5.2-24.7)	24.2 (9.7-119.8)	<0.001
Creatinine (mg/dl)	0.83 (0.56-1.46)	0.95 (0.44-4.78)	<0.001
Total Bilirubin (mg/dl)	1.37 (0.22-4.39)	3.53 (0.51-32.58)	<0.001
Direct Bilirubin (mg/dl)	0.45 (0.12-1.5)	1.29 (0.22-11.79)	<0.001
ALT (U/L)	31 (2-186)	52 (4-252)	<0.001
AST (U/L)	36 (13-112)	65 (11-458)	<0.001
Total Protein (g/dl)	6.9 (4.8-8.5)	6.2 (4.8-8.1)	<0.001
Albumin (g/dl)	3.8 (2.2-4.7)	2.9 (1.4-4.3)	<0.001
Sodium (mmol/L)	137 (119-143)	134 (118-145)	<0.001
Potassium (mmol/L)	3.6 (2.5-4.9)	3.6 (2.6-4.9)	
Chloride (mmol/L)	102 (95-110)	99 (85-109)	<0.001

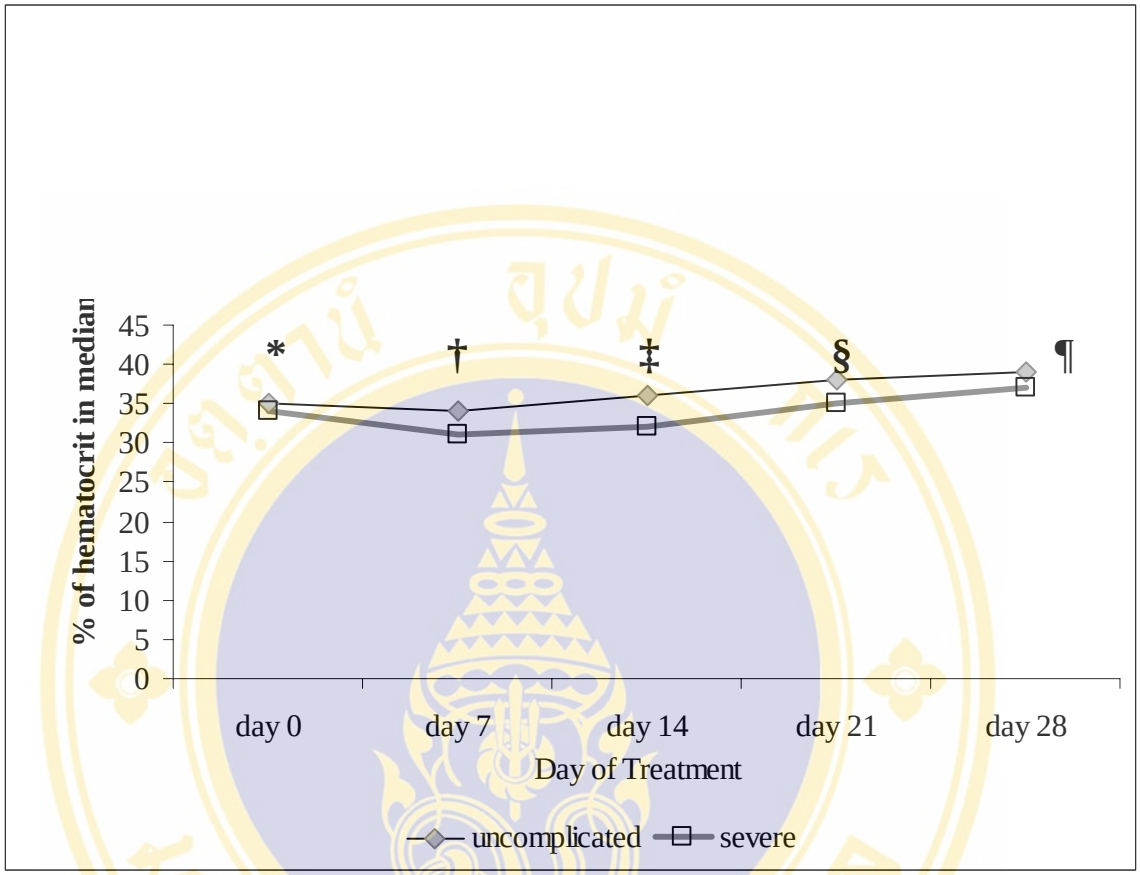

Figure 1. Median hematocrit value in uncomplicated and severe malaria patients in percentage from Day 0 to Day 28. (* p=0.029 , †, ‡, §, ¶= p<0.001)

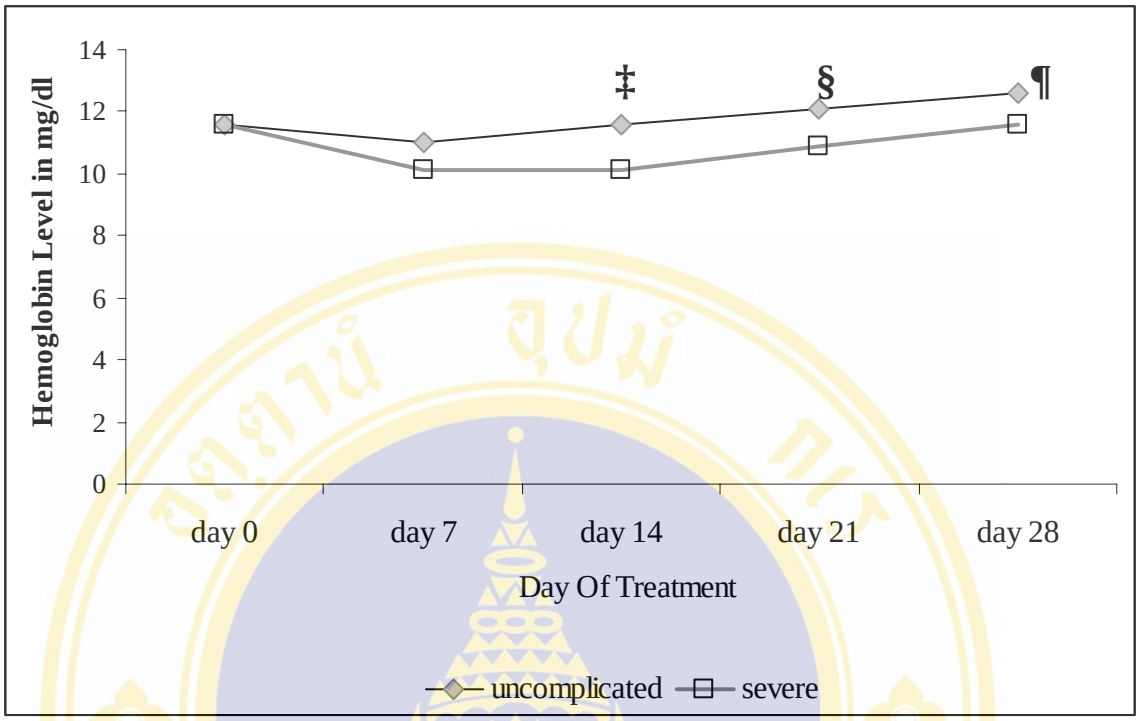

Figure 2. Median Hemoglobin value in uncomplicated and severe malaria patients in mg/dl from Day 0 to Day 28. **(‡, §, ¶** = p<0.001)

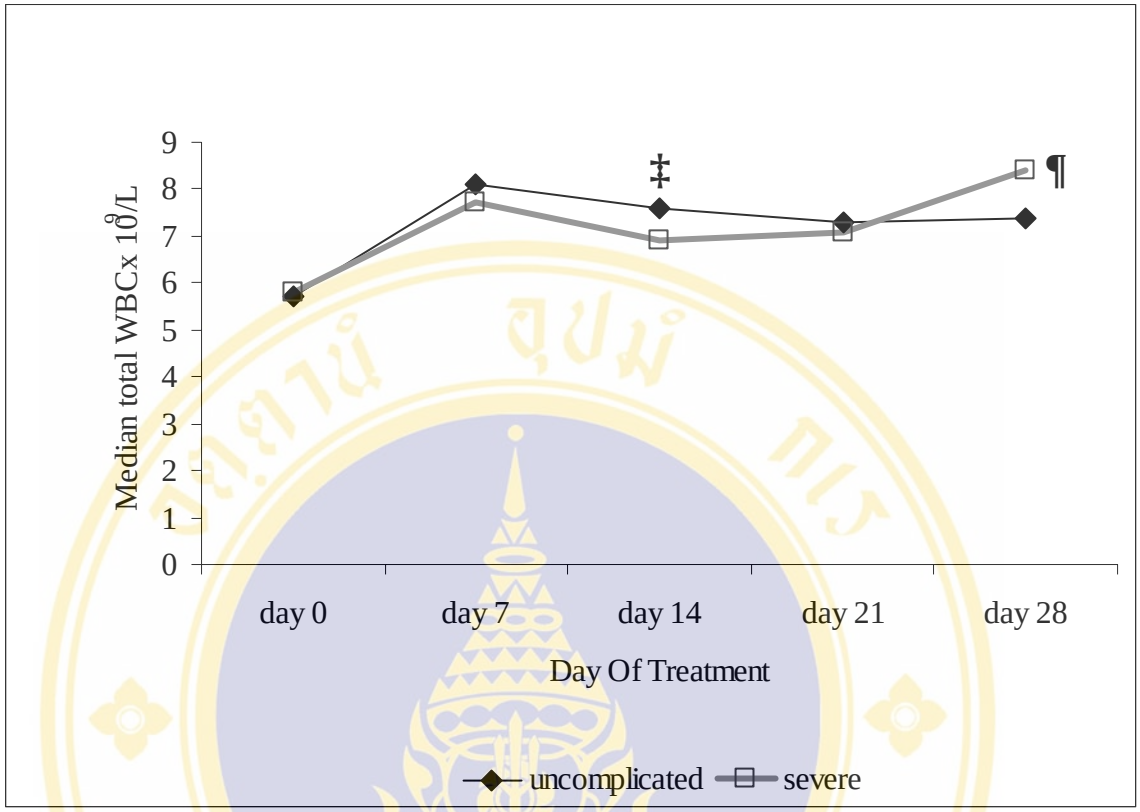

Figure 3. Median total WBC in relation to 10^9/ L in uncomplicated and severe malaria patients from Day 0 to Day 28. (‡ p=0.043, ¶ p=0.013)

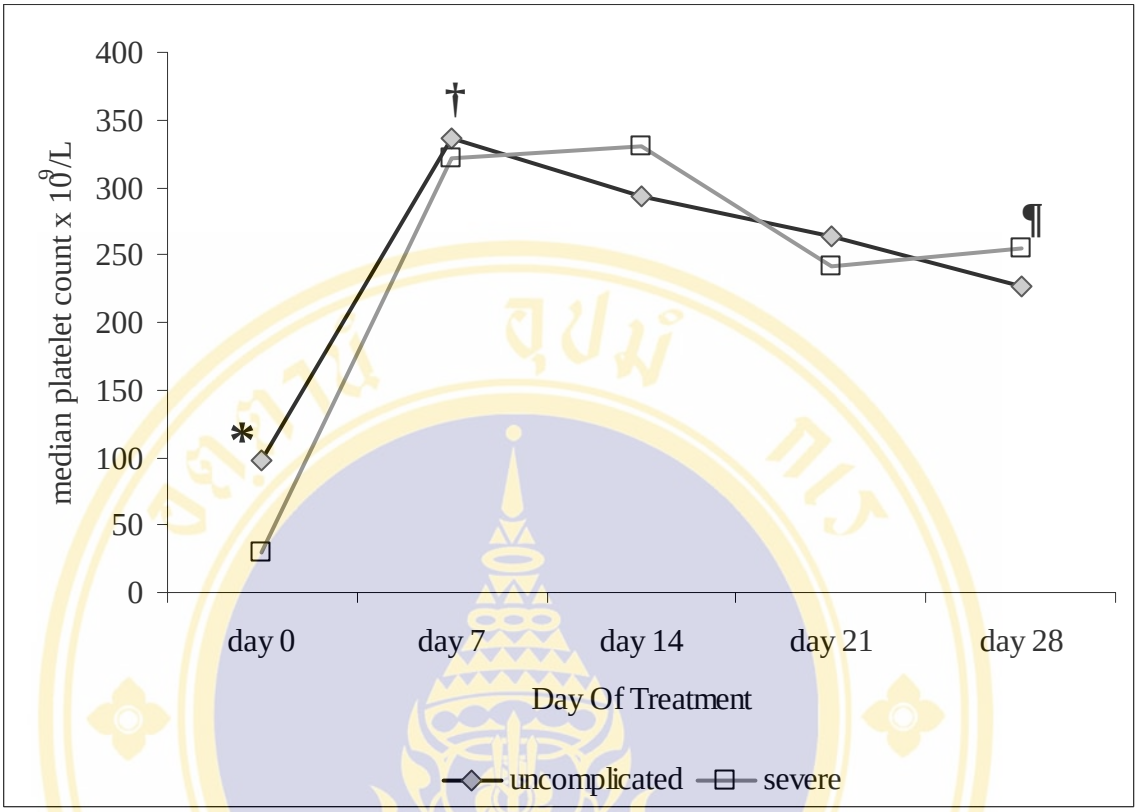

Figure 4. Median platelet count in relation to 10^9/L in uncomplicated and severe malaria patients from Day 0 to Day 28. **(*** p<0.001, † p=0.022, ¶ p=0.004)

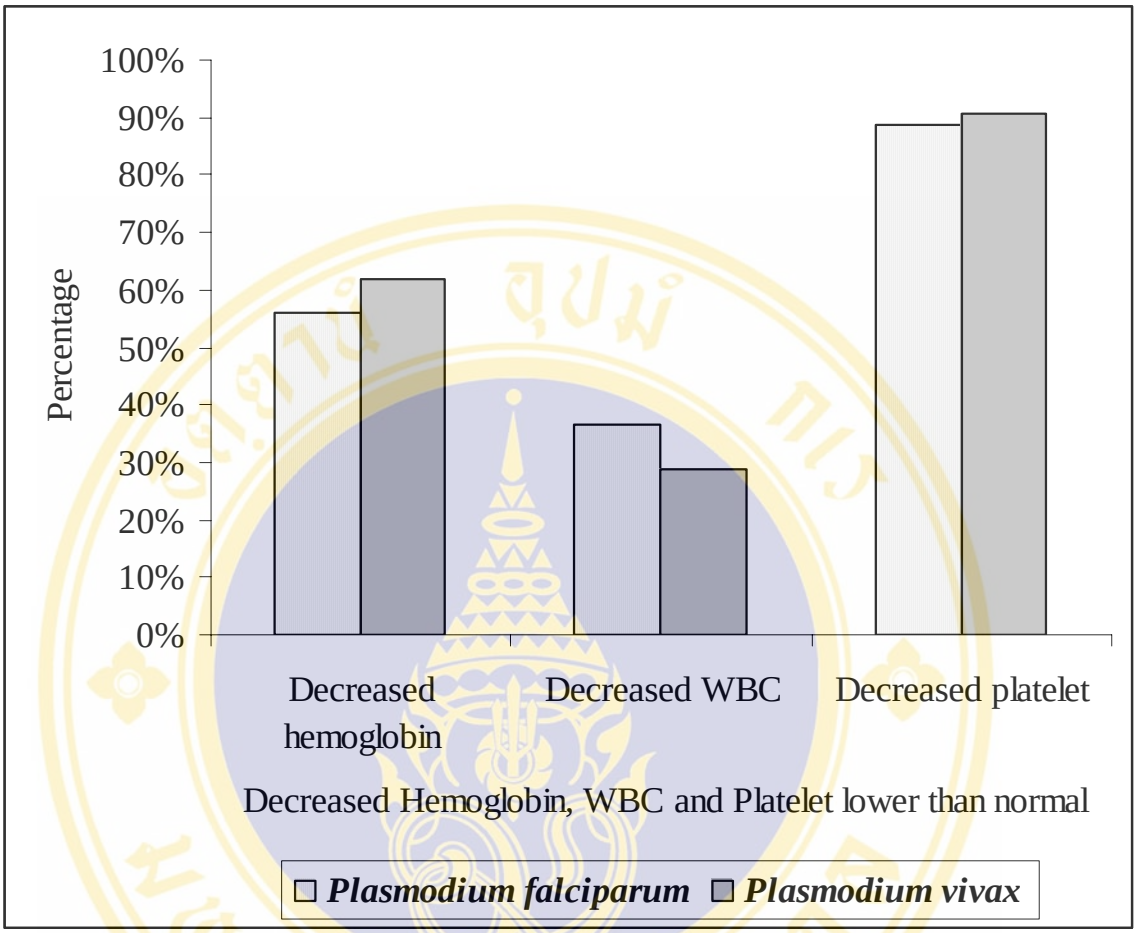

Figure 5. Comparison of percentage of low hemoglobin level, total WBC and platelet level than normal in *Plasmodium falciparum* and *Plasmodium vivax* infection.

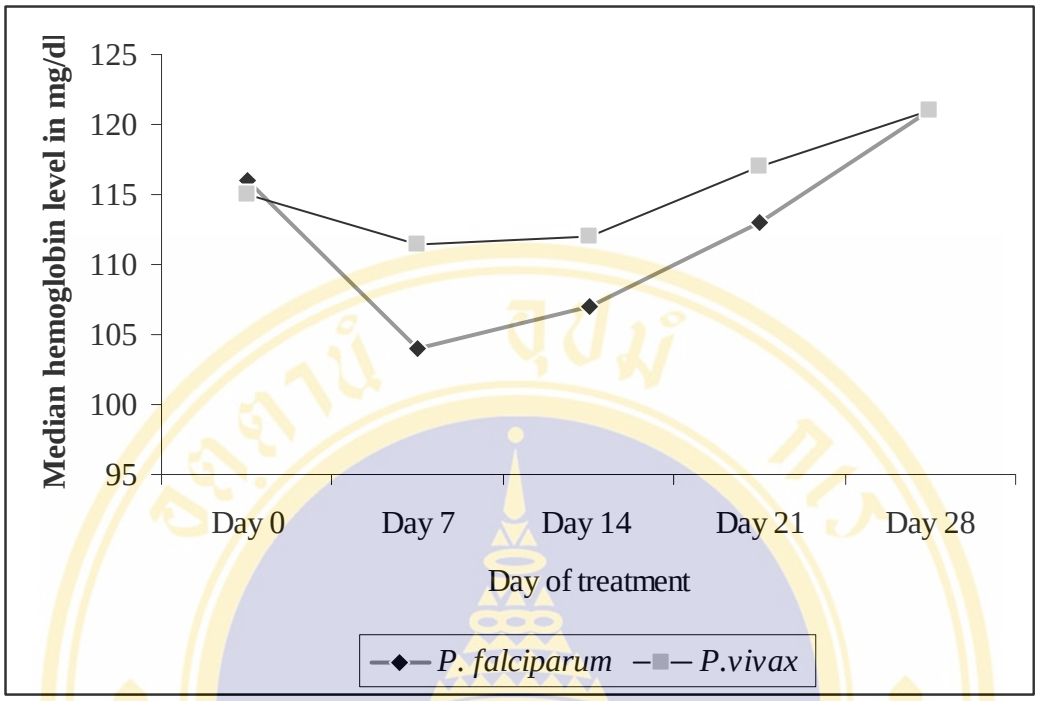

Figure 6. Result of median hemoglobin level in *Plasmodium falciparum* and *Plasmodium vivax* infection in mg/dl from Day 0 to Day 28.

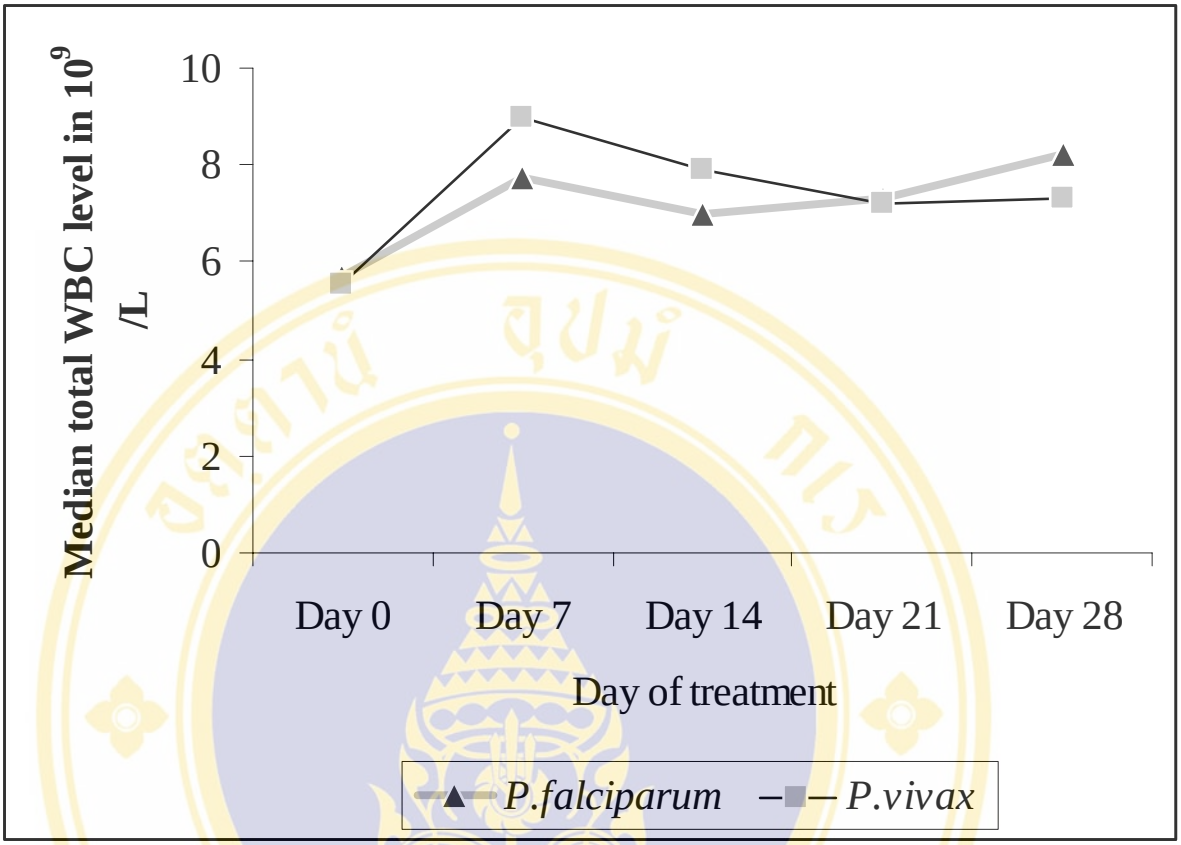

Figure 7. Result of median total WBC level in *Plasmodium falciparum* and *Plasmodium vivax* infection in 10^9/L from Day 0 to Day 28.

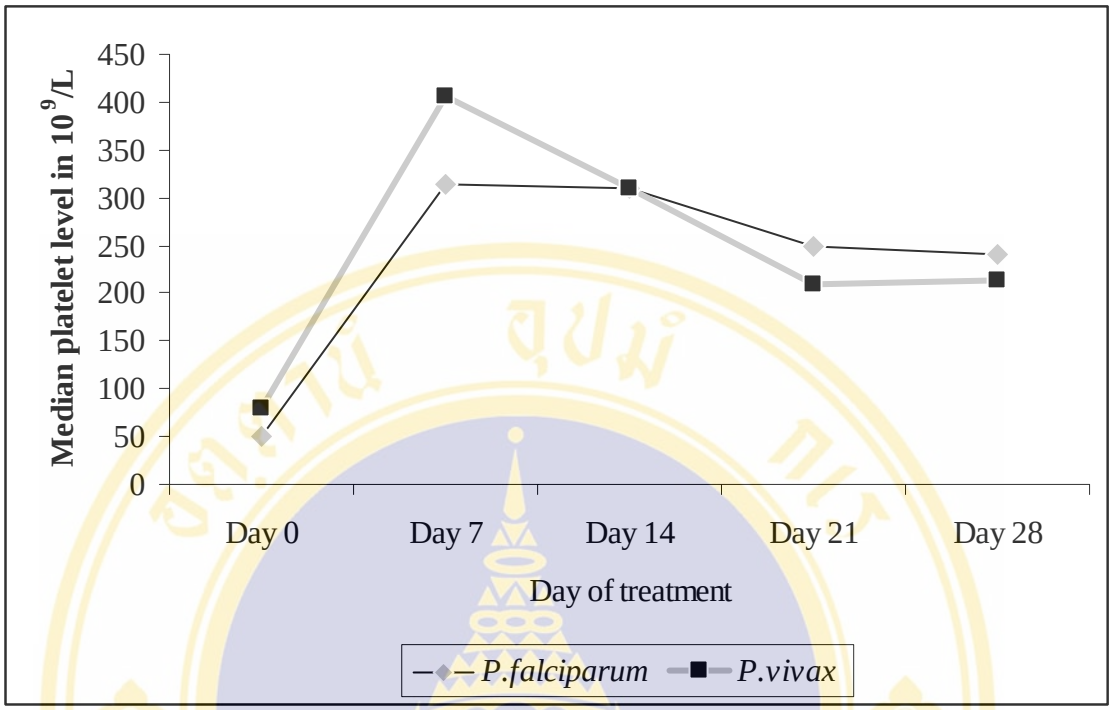

Figure 8. Result of median platelet count in *Plasmodium falciparum* and *Plasmodium vivax* infection in 10^9/L from Day 0 to Day 28.

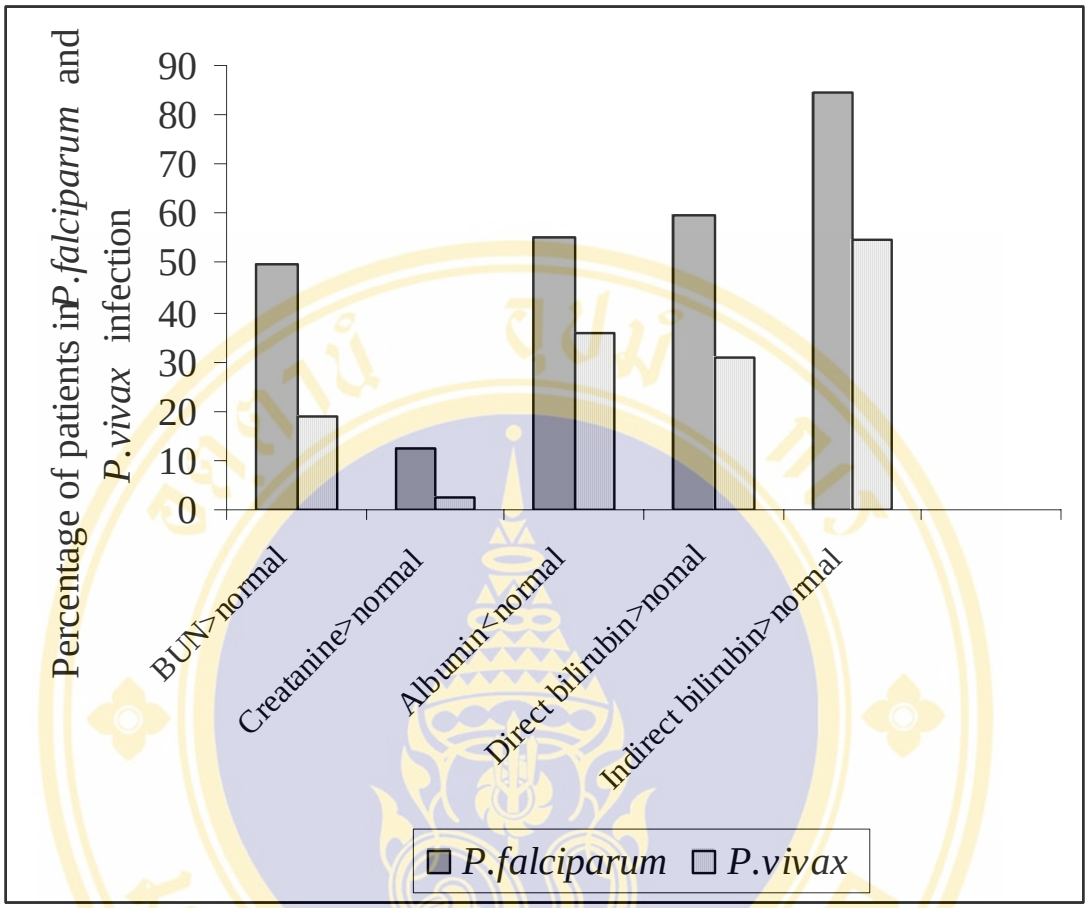

Figure 9. Percentage of patients in *Plasmodium falciparum* and *Plasmodium vivax* infection with high BUN and creatanine level,low albumin level with high direct bilirubin and indirect bilirubin level compared to normal value on baseline.

CHAPTER VI

DISCUSSION

Hematological abnormalities are considered a common finding of malaria, and reported to be most pronounced in *P.falciparum* infection. In this study, 204 patients were participated; 106 (52%) uncomplicated patients and 98 (48%) severe patients. When we observed the significance of ethnic group, Mon and Karen people were common and it was because all the patients who participated in the study came from the Myanmar-Thai border area especially these border areas are quite close to Mon and Karen State. Hence, most of the people from these States came and worked in Kanchanaburi, Tak, Ratchaburi provinces. Naturally, most of them were men and so there was male predominance in uncomplicated and severe groups. When the residence provinces were compared, there are no significant difference for the patients from Tak and Kanchanaburi regarding the severity of disease whereas there was big difference in patients from Ratchaburi group where 16 uncomplicated patients with one severe patient. The reason for this difference could be whether these patients had some immunity to malaria or previously they got chemoprophylaxis or the less number of patients from this province (only17) participated compared to other provinces where 115 patients from Tak and 72 patients from Kachanburi. The result for previous history of malaria showed that 43 (40.6%) of uncomplicated patients had experience about the disease before which was more than that of for other group (18, 18.4%) ($p = 0.002$). It meant that it could be relapse or those patients had previous attack and those repeated infection in them eventually develop a degree of immunity which leads to only relatively mild attacks. In those individuals without previous exposure to malaria such as migrants were more prone to get severe acute disease. The next reason could be those patients had experience about the disease so they had some knowledge to take chemoprophylaxis but it could not cover the dose which could protect from the disease actually. So, it led to uncomplicated form in stead of having complications.

All baseline clinical findings except body temperature were significant between uncomplicated and severe groups ($p<0.001$) because they were more aggressive in severe forms. When we studied the malaria species, falciparum infection was the most common 159 (77.93%) and vivax was the second most 42 (20.6%). It is known that falciparum and vivax infections are common in South-East Asia. Moreover, as we know that these two infections are common for hepatosplenomegaly especially falciparum infection. In this research, 79 (49.7%) and 11 (6.9%) of falciparum infected patients had enlarged liver and spleen respectively. Some of them had both liver and spleen enlargement. Regarding the complication of malaria, the complications such as cerebral malaria, acute renal failure with or without dialysis, hyperparasitemia and anemia were mainly contributed to falciparum infected group.

Additionally, the baseline hemoglobin level of female patients was lower that of for male in both uncomplicated and severe group which indicated that the nutritional deficiency and facing monthly menstrual period could be more risk to get low hemoglobin level in female.

When we did the analysis to know the source of anemia, it meant that we did the correlation between baseline hemoglobin level with that of indirect and direct bilirubin level and it showed that for both groups, there were more patients for high indirect bilirubin level than direct bilirubin. It verified that there was low hemoglobin level with increased level of indirect bilirubin level which could be due to massive hemolysis more than the conjugation that liver could do or the liver was impaired and so it unable to do all conjugation process or due to both mechanisms. However, the relationship between baseline hemoglobin level and direct bilirubin level was significant in severe patients and it was clear that there was some hepatocellular injury in severe cases. For uncomplicated group, there was low hemoglobin level with high unconjugated form with normal median value of ALT and AST level throughout the study period showed there was no liver failure and high indirect bilirubin might be due to hemolysis. For renal failure, we compared the baseline BUN level and creatanine level in both groups. There were more severe patients with BUN and creatanine level more than normal (>19 mg/dl, >1.4 mg/dl respectively) compared to uncomplicated group but there were 5 severe patients whose BUN and creatianine level were very high (> 60 mg/dl, > 3 mg/dl respectively) to say that those levels for severe malaria.

Hence, it could be said that there was association between severe malaria and acute renal failure. Those 5 patients were treated by hemodialysis. All of them were falciparum infected patients and it is clear that falciparum infection is mainly responsible for severe malaria. The baseline hemoglobin and albumin level for both complicated and uncomplicated group were significant but low albumin level was more pronounced in female patients. The significance of low level of albumin could be hepatic injury or it ran out by combination of unconjugated bilirubin to dissolve in water. In this study, there were increased in direct bilirubin level and decreased albumin level were consistent with the study of L.H Camacho (L.H Camacho *et al.*, 1998) They said that it may be due to hepatocellular injury or/and outpouring of inflammatory cytokines during the disease process.

As we know that parasite clearance time and fever clearance time are important for evaluation of disease outcome, they are studied for uncomplicated and severe patients. Naturally, patients with fewer parasites took shorter period to clear all the parasites and quicker time to get resolution from fever. Uncomplicated patients had fewer parasites in their body and thus, they had shorter parasite clearance time and fever clearance time. For hemoglobinopathies and red cell enzyme deficiency, there was no relationship between them and severity of the disease except Hemoglobin E homozygous where it was statistically significant but the sample size was too low for both groups to compare each other (5 for uncomplicated and 0 for severe) so we could not say that it was really significant or not. Moreover, it was proved that all hemoglobinopathic patients and G6PD deficient patients had history of previous experience of malaria and some complications. Thus, generally, it was difficult to prove that hemoglobinopathies and red cell enzyme deficiency can protect malaria infection to a certain degree.

When hematological data were studied for the whole study (Day 0 – Day 28), the median hemoglobin level of uncomplicated patients showed less than 12 mg/dl in first week but it started increased gradually in the subsequent weeks (up to Day 28) and finally it touched to normal whereas in severe patients, it still low up to 10 mg/dl in Day 14 and then it started up and then until Day 28, it cannot touch the level 12 mg/dl.

We found that more than 70 % of patients in both groups encountered parasitic infection (worm infestation) together with malaria but we could not say that anemia was because of it as we could not calculate the parasite density and so the degree of its affect on hemoglobin level was difficult to mention. Generally, it was quite clear that uncomplicated patients had better and quicker chance to get normal hemoglobin level than severe patients. The same effect of hematocrit level in both groups as hemoglobin level did. Anemia was normochromic normocytic anemia (6 for male and 14 for female) and more common in female. The pathogenesis of anemia in malaria is quite complex, multi factorial and incompletely understood. It is thought to result from a combination of hemolysis of parasitized red blood cells, accelerated removal of both parasitized and innocently unparasitized red blood cells, depressed as well as ineffective erythropoiesis with dyserythropoetic changes and anemia of co-infection with other parasites (such as worms). Other factors contributing to anemia in malaria include decreased red blood cell deformability, splenic phagocytosis and / or pooling, so they have an increased rate of clearance from the circulation. In this study, there was some evidence of hypoproliferative erythropoiesis after the malaria treatment in both uncomplicated and severe patients in first week and it may be due to on-going inflammation, reduced number of erythroid progenitors or presence of concomitant nutritional deficiency, hemoglobinopathies or red cell enzyme deficiency. Cytokines may be responsible for suppression of erythropoiesis (Camacho L.H *et al.*,1998).

When we compared the level of hemoglobin with malaria species, falciparum infection was mainly responsible for low level of hemoglobin and vivax infection is the second most cause.

The media values for total WBC count were analyzed for the whole study; it showed that low level of WBC but more than 5 x 10^9/l in baseline for both groups. It is not easy to describe the exact mechanism but it could be due to the destruction of parasitized white blood cell by spleen or immune reaction or due to bone marrow suppression by cytokines. After one week, it went up for both groups to a certain level (~8 x 10^9/l) and then it ran down again on Day 14 and there was significance between uncomplicated and severe group. We don’t know exactly why it happened and when we observed the absolute number of differential count, we found that segment and eosinophil level in Day 14 showed difference in uncomplicated and severe group. The

mechanism of that difference after the malaria treatment could not be explained. We hope that future studies will provide more information. All the changes of total WBC in the whole study period occurred within the normal level (5 – 10 x 10^9 / l). The possible mechanisms for persistent lowering of segment could be continuous suppression of segment production by inflammatory products of the disease and / or still persist of splenic clearance. To explain the exact mechanism, we need further study and investigations. Besides WBC, low platelet count found consistently for both uncomplicated and severe patients as baseline but for severe patients, the level was very low and it was less than 50 x 10^9 /L which showed significance. However, within one week after getting treatment, the level shot up to nearly (350 x 10^9/l) for both groups and there was still significance between two groups. For uncomplicated patients, it went down linearly up to upper normal (250 x 10^9 / l) in Day 28 but for severe patients, thrombocytosis still persist up to Day 14 and then it gradually down up to normal in Day 28. The finding of thrombocytopenia on first day agreed with other study (Erhart L.M *et al*., 2003)The mechanism could be peripheral destruction by macrophages or much platelet adhesion to both parasitized and nonparasitised RBC during the disease process or premature platelet destruction by oxidative stress. It was said that disseminated intravascular coagulation could lead to thrombocytopenia but it is not relevant to our study as there was no DIC patient in present study.

CHAPTER VII

CONCLUSION

In conclusion, the hematological aspects of malaria infection can give rise interested aspect out of other changes in malaria. In this study, we could find that there are many relationships between malaria and hematological changes. Plasmodium falciparum and Plasmodium vivax infection mainly contributed to most of the changes such as low hemoglobin level and platelet level in baseline. We could not do analysis for *P.malariae* and *P.ovale* as limited sample size to carry out analysis. However, we could do a lot of study of correlation between uncomplicated and severe malaria in hematology. In addition, we found that the relationship of hemoglobinopathies and G6PD deficiency with severity of malaria. Some of our findings were consistent with previous studies although some were against to others. Principally, anemia on baseline, persistent lowering of neutrophil and baseline thrombocytopenia followed by thrombocytosis are the main changes in this study. Worm infestation in 70% of patients created the confounding factor for the study. Hence, it would be more beneficial to study more patients with different types of malaria and different hemoglobinopathies with prospective studies and have chance to do more investigations to exclude other causes that can affect to hematological parameters.

As a conclusion, these findings in this study are sincerely expected to be useful to many clinicians and health care personnel to detect and have awareness of the hematological changes of malaria to improve the management to get better clinical outcome.

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APPENDIX

APPENDIX

CASE RECORD FORM

Patient Data*:*

Patient Code : …….

Date of admission: …../…../…..

Gender: …….

Weight: …….kg

Height: …….cm

Residence Province: ……..

Nationality: ……..

Occupation: ……..

History*:*

No. of previous malaria infections: …….

Origin of current infection: ……..

Days of fever PTA: …….days

Alcohol: …….

Smoker: …….

(0 = no, 1 = yes, 9 = data missing))

Clinical findings:

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28
weakness						
Chills/rigor						
Dizziness						
Abdominal pain						
Diarrhea						
Anorexia						
Nausea						
Vomiting						
Palpitations						
Other						
Other						

(0 = no, 1 = mild; 2 = moderate, 3 = severe, 9 = data missing)

Physical examination on admission:

Temperature: …….°C

Pulserate: ……./min

Blood pressure: ……./…….

Anemia: …….yes □ no □

Jaundice: …….yes □ no □

Dehydration: …….yes □ no □

Neurological manifestation: ……. (if abnormal, specify……)

Liverenlargement yes □……..cm

no □

Spleenenlargement: yes □……..cm

no □

Malaria:

P.falciparum ☐

P.vivax ☐

P.malariae ☐

P.ovale ☐

Complications: …….CM

…….ARF dialysis no ☐

yes ☐

day after treatment:…….

…….pulmonary edema

duration:…….

…….respiratory distress

…….severe normocytic/ hypochromic anemia

…….hyperparasitemia

…….hypoglycemia

…….circulatory collapse (shock)

…….spontaneous bleeding/ DIC

…….repeated generalized convulsions

…….acidosis

Parasite count on day 0: …….MP/μl

Parasite clearance time: …….hrs

Fever clearance time: …….hrs

Hematology*:*

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 29
WBC						
RBC						
Hb g/dl						
Hct.						
MCV fl						
MCH pg						
Plt						
G6PD						
Band %						
Segm %						
Eos %						
Baso %						
Lym %						
Mo %						
RDW %						

WBC x 10^9/l; RBC x 10^{12}/l; Plt x 10^9/l;

(9 = data missing)

Hb-Typing*:*

A: …….%

A2: …….%

HbH: …….%

HbE: …….%

(9 = data missing)

Interpretation:……

Blood transfusion*:*

transfusion:…….

(0 = no, 1 = whole blood, 2 = packed red cells, 3 = plasma)

Units:…….

day after admission:…….

Plt transfusion:…….

(0 = no, 1 = yes)

Units:…….

day after admission:…….

(9 = data missing)

Chemistry:

	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28
Glu mg/dl						
BUN mg/dl						
Cr mg/dl						
D Bili mg/dl						
T Bili mg/dl						
Prot g/dl						
Alb g/dl						
Glob g/dl						
Alk P U/l						
AST U/l						
ALT U/l						
Na mmol/l						
K mmol/l						
Cl mmol/l						

Summary:

Type of malaria:……

Parasite clearance time:…….

Fever clearance time:…….

Complications:……..

……

…….....

Blood transfusion: no □

yes □

Hb-Typing (interpretation)……..

G6PD status:…….

(0 = normal, 1 = deficiency)

Patient outcome: survived □

died: □

…….days after treatment

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