DYANAMICITY OF PLATELETS IN PATIENTS WITH PLASMODIUM FALCIPARUM MALARIA

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DYANAMICITY OF PLATELETS IN PATIENTS WITH PLASMODIUM FALCIPARUM MALARIA

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ABSTRACT

The purpose of this study was to find the association of platelet count changes in both uncomplicated and severe malaria patients. A retrospective study was conducted of 220 patients, age 15 years and older who had undergone anti malarial treatment.

In this study, there were 47 Thai (21.4%), 35 Hmong (15.9%), 6 Burmese (2.7%) and 22 Karen (10%) in the uncomplicated group and 37 Thai (16.8%), 34 Hmong (15.5%), 15 Burmese (6.8%) and 24 Karen (10.9%) in severe group. The is less severe in patients who have a history of malaria infection. Thrombocytopenia was found in almost all of falciparum infections both uncomplicated and severe. 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. The results revealed no association between total white blood cell (WBC) count on the day of presentation and the severity of the malaria. Total WBC (White Blood Cell) count was normal throughout the study period. In addition, thrombocytopenia was first presented at baseline, but returned to a higher level than normal after treatment and then became normal in both groups by the end of study. In this study, all baseline hematological data levels were lower than normal in general, which may be due to hemolysis, hypo proliferative 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 cases because of the aggressiveness of the disease.

Thrombocytopenia can implicate complications, but platelet transfusions are generally not required because of the patients recover quickly. The presentation of anemia on day 0 is also not suitable for assessing the severity of malaria.

KEY WORDS : MALARIA/ TROMBOCYTOPENIA/ ANEMIA

66 pages.

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LIST OF ABBREVIATIONS

Abbreviation or Symbol	Term
CBC	complete blood count
cm.	Centimeter
ECG	electrocardiogram
G6PD	gluscose 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

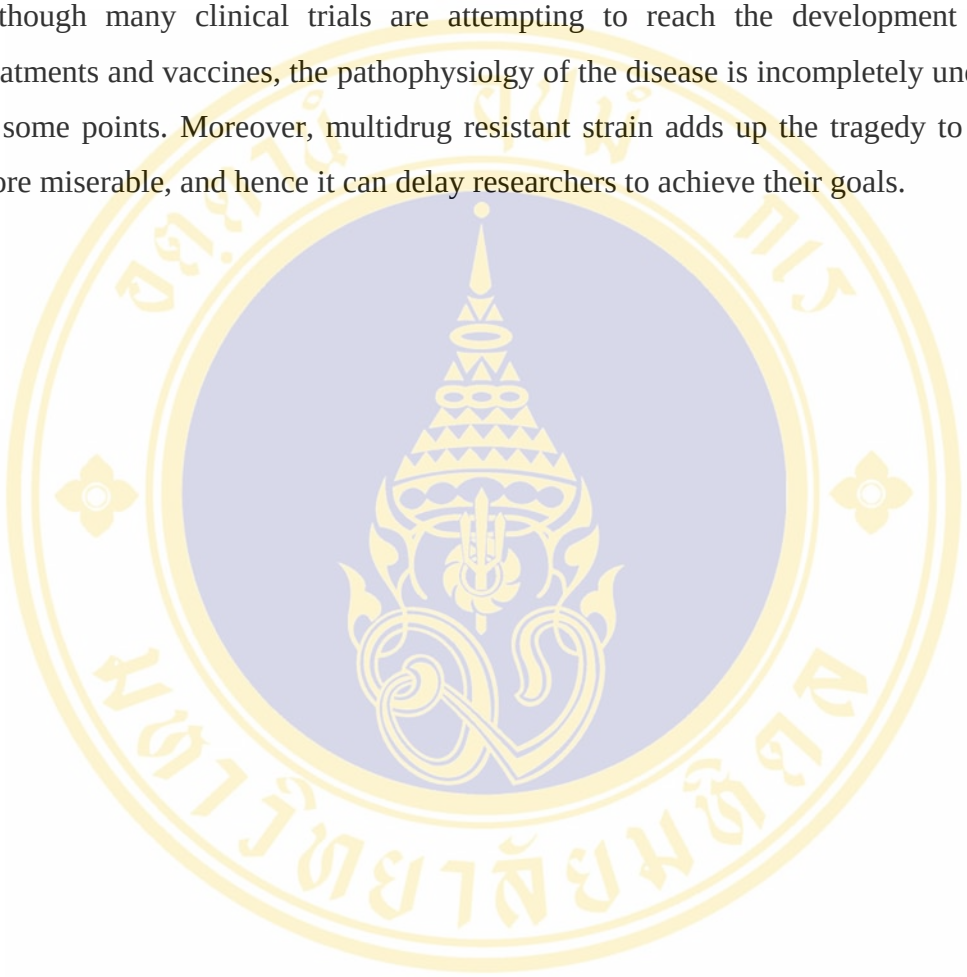
LIST OF ABBREVIATIONS (cont.)

Abbreviation or Symbol	Term
mEq	miliequivalent
min	minute
MP	malaria parasite
Pg	picogram
P. falciparum	Plasmodium falciparum
P. viavx	Plasmodium vivax
P. malariae	Plasmodium malariae
P. ovale	Plasmodium ovale
PTA	piror 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 parasite 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 America. Approximately, 5% of the world population is infected and estimated one million deaths each year. 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. The Anopheles mosquitoes (female) transmit the disease by biting to human and with the saliva of mosquitoes; the parasite enters into the human body via blood stream and precede 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.vaivx*, *P.ovale*, *P.malariae*, which can cause benign malaria. In Africa, the most predominant one is *P.falciparum* malaria where as *P.vivax* is more common in central and parts of south Africa, 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 death. The common presentation of uncomplicated *P.falciparum* is fever, headache, fatigue, malaise, aches and pain, sometime 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, G6PD deficiency and malaria immunity. Although many clinical trials are attempting to reach the development of new treatments and vaccines, the pathophysiolgy of the disease is incompletely understood in some points. Moreover, multidrug resistant strain adds up the tragedy to become more miserable, and hence it can delay researchers to achieve their goals.



CHAPTER II
OBJECTIVE

To find the dynamic changes of platelet count before, during and after treatment of *P. falciparum* malaria.

CHAPTER III
LITERATURE REVIEW

Malaria remains a devastating global health problem. Worldwide, estimated 300–500 million people contract malaria each year, resulting in 1.5–2.7 million deaths annually (Muentenar et al., 1999; Sachs et al., 2002). Because of the increase in global travel to and immigration of people from areas endemic for malaria, the incidence of imported cases of malaria in developed countries has risen. Approximately 10000–30000 travelers from industrialized countries are expected to contract malaria each year (Kain et al., 1998).

In addition, drug-resistant *Plasmodium falciparum* malaria continues to spread and at present involves almost all areas of the world. An increasing number of travelers are exposed to drug-resistant plasmodia. Malaria is found in many parts of the world but endemic in Southeast Asia, Latin America and sub-Saharan Africa. Malaria continues to pose a major public-health threat to many countries in Africa. The launch of Roll Back Malaria (RBM) therefore was regarded as timely by many. International public health initiatives such as RBM, aimed at reducing continental burdens of malaria, require an understanding of contemporary malaria distribution, risk and burden. Several attempts have been made to explore continental malaria distribution and disease burden using climate models and malariometric data gathered from the literature. There is increasing evidence, however, that the relationship between the frequency of infection and disease outcome is complex and control options should be selected accordingly.

Malaria is caused by obligate intraerythrocytic protozoa of the genus *Plasmodium*. It was discovery by Charles Louis Alphonse Laveran, a French army surgeon stationed in Constantine, Algeria. He was the first to notice parasites in the blood of a patient suffering from malaria on the 6th of November 1880. For his discovery, Laveran was awarded the Nobel Prize in 1907. Humans can be infected with one (or more) of the following four species: *P. falciparum*, *P. vivax*, *P. ovale*,

and *P. malariae. P. falciparum* is found particularly in Sub-Saharan Africa and Melanesia (Papua New Guinea and the Solomon Islands), whereas *P. vivax* is found mainly in the Indian subcontinent, Central and South America, North Africa and the Middle East. *P. ovale* is found almost exclusively in West Africa, and the relatively few cases of *P. malariae* infection occur mainly in Africa. Human malaria is commonly caused by *Plasmodium falciparum*, *P. vivax, P. malariae,* and *P. ovale*. However, a large focus of human infections with the simian malaria parasite, *P. knowlesi*, has recently been reported in Malaysian Borneo, and single case reports of infections acquired in Thailand and Myanmar, have been documented (Singh et al., 2004; Jangwatiwes et al., 2004; Zhu et al., 2006). The diagnosis of *P. knowlesi* in humans may be missed by microscopy since the early blood stages of *P. knowlesi* morphologically resemble *P. falciparum*; the mature blood stages and gametocytes are similar to those of *P. malariae.*

Reports of human *P. knowlesi* infections are confined to Southeast Asia, particularly Malaysia, but there are also reports on the Thai-Burmese border (Singh et al., 2004; Jangwatiwes et al., 2004; Chin et al., 1965). A fifth of the cases of malaria diagnosed in Sarawak, Malaysian Borneo are due to *P. knowlesi*. *P. knowlesi* infection is normally considered an infection of long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaques, but humans who work at the forest fringe or enter the rainforest to work are at risk of being infected with *P. knowlesi*. The mosquito *Anopheles latens* is attracted to both macaques and humans and has been shown to be the main vector transmitting *P. knowlesi* to humans in the Kapit Division of Sarawak, Malaysian Borneo (Vythilingam et al., 2006). Within the monkey population in Peninsular Malaysia, *Anopheles hackeri,* is believed to be the main vector of *P. knowlesi* although *Anopheles hacker'*is capable of transmitting malaria to humans, it is not normally attracted to humans and therefore cannot be an important vector for transmission (Wart et al., 1961).

'Airport malaria' occurs when infected mosquitoes are brought from endemic areas on planes; cases have occurred during hot summers in non-endemic areas, when infected individuals have passed the infection to the local mosquito population. Mosquitoes could transmit the malaria parasite was first discovered by Ronald Ross, a British officer in the Indian Medical Service on August 20th, 1897. He was the first to

demonstrate that malaria parasites could be transmitted from infected patients to mosquitoes. In further work with bird malaria, Ross showed that mosquitoes could transmit malaria parasites from bird to bird. This necessitated a sporogonic cycle (the time interval during which the parasite developed in the mosquito). Because of him the problem of malaria transmission was solved. For this discovery Ross was awarded the Nobel Prize in 1902. Plasmodia are primarily transmitted by the bite of an infected female *Anopheles* mosquito, but infections can also occur through exposure to infected blood products (transfusion malaria) and by congenital transmission. In industrialized countries, most cases of malaria occur among travelers, immigrants, or military personnel returning from areas endemic for malaria (imported malaria). Exceptionally, local transmission through mosquitoes occurs (indigenous malaria).

Among patients with unexplained fever or clinical deterioration who have returned from an endemic area within the past few years, malaria must be included in the differential diagnosis. The evaluation of such cases should always include taking a comprehensive travel history. Delays in recognition and appropriate treatment of malaria increase morbidity and mortality (Kain et al., 1998). Here, the clinical manifestations, laboratory findings, diagnosis, and treatment of severe malaria are reviewed.

When the infected anopheline mosquito takes a blood meal, sporozoites are inoculated into the bloodstream. Within an hour sporozoites enter hepatocytes and begin to divide into exoerythrocytic merozoites (tissue schizogony). For *P. vivax* and *P. ovale*, dormant forms called hypnozoites typically remain quiescent in the liver until a later time; *P. falciparum* does not produce hypnozoites. Once merozoites leave the liver, they invade erythrocytes and develop into early trophozoites, which are ring shaped, vacuolated and uninucleated. Once the parasite begins to divide, the trophozoites are called schizonts, consisting of many daughter merozoites (blood schizogony). Eventually, the infected erythrocytes are lysed by the merozoites, which subsequently invade other erythrocytes, starting a new cycle of schizogony. The duration of each cycle in *P. falciparum* is about 48 hours. In non-immune humans, the infection is amplified about 20-fold each cycle. After several cycles, some of the merozoites develop into gametocytes, the sexual stage of malaria, which cause no symptoms, but are infective for mosquitoes (Garcia et al., 2001).

In nonimmune individuals with *P. falciparum* infection, the median pre-patent period (time from sporozoite inoculation to detectable parasitemia) is 10 days (range 5–10 days), and the median incubation period (time from sporozoite inoculation to development of symptoms) is 11 days (range 6–14 days). The incubation period may be significantly prolonged by the level of immunity acquired through previous exposures, by antimalarial prophylaxis, or by prior partial treatment, which may mitigate, but not prevent the disease (Taylor et al., 2000). Most nonimmune travelers develop symptoms of *falciparum* malaria within 1 month of departing from a malaria-endemic area (median 10 days) there have been reports of *falciparum* malaria presenting up to 4 years later (White et al., 2003). For non *falciparum* malaria the incubation period is usually longer (median 15–16 days), and both *P. vivax* and *P. ovale* malaria may relapse months or years after exposure due to the presence of hypnozoites in the liver. The longest reported incubation period for *P. vivax* is 30 years (White et al., 2003).

The clinical symptoms of malaria are primarily due to schizont rupture and destruction of erythrocytes. Malaria can have a gradual or a fulminant course with nonspecific symptoms. The presentation of malaria often resembles those of common viral infections; this may lead to a delay in diagnosis (Murphy et al., 2001). The majority of patients experience fever (>92% of cases), chills 79%, headaches 70%, and diaphoresis 64% (D'Acremont et al., 2002).

With the first attack of *P.falciparum*, fever is usually irregular rather than occurring with a regular, repeating pattern as seen with a tertian fever in subsequent attacks, and there are usually no relapses unlike with *P.ovale* and *P.vivax* where hypnozoites are formed (A Colour Atlas of Tropical Medicine and Parasitology. 4th Ed). Fever is not always present, and rigors may or may not be present. The temperature may rise above 41°C, over several days, and the fever is produced as the schizonts mature, at 48 hour intervals usually for *P.falciparum*. The different malarias produce fevers of different frequency, depending on how long it takes to complete shizogony in erythrocytes. Malaria is especially dangerous to pregnant women and small children. Severe and complicated malaria is usually caused by delay in treating an uncomplicated attack of *P.falciparum*.

The patient will complain of headache, fever and aches and pains all over the body, and diarrhea and abdominal pain are sometimes present. Spleen and liver are often palpable on clinical examination. This may be misdiagnosed as influenza in non endemic areas, and, unless treated promptly, the clinical picture can deteriorate rapidly (Management of severe and complicated malaria: a practical handbook, WHO). A patient with severe and complicated malaria will often present with impaired consciousness, weakness, and jaundice. Other complications are cerebral malaria (unrousable coma), generalized convulsions, normocytic anaemia, renal failure, hypoglycemia, fluid, electrolyte and acid-base disturbances, pulmonary oedema, circulatory collapse, shock, disseminated intravascular coagulation, hyperpyrexia, hyperparasitaemia, and malarial haemoglobulinurea. These features may occur singly or in combinations.

As to whether the Plasmodium parasites cause their mosquito hosts such discomfort and morbidity is a matter of debate, with several contrasting opinions stressed. It does seem that mosquitoes that are carrying the malaria parasite do experience decreased life expectancy and higher mortality rates than their non infected counterparts. Back in the human, the immune response is very important. Cell mediated immunity plays an active role and, in immune individuals, a large increase in IgG production (specific to antigens such as the circumsporozoite proteins on the sporozoites) occurs. Vaccines are currently being developed against three stages of the parasite: gametocytes, sporozoites, and intra-erythrocytic merozoites. The most important part of host defense seems to be antibody production, hence non-immune individuals visiting endemic areas are very vulnerable.

Other common symptoms include dizziness, malaise, myalgia, abdominal pain, nausea, vomiting, mild diarrhea, and dry cough. Physical signs include fever, tachycardia, jaundice, pallor, orthostatic hypotension, hepatomegaly, and splenomegaly. Clinical examination in nonimmune persons may be completely unremarkable, even without fever.

Almost all severe forms and deaths from malaria are caused by *P. falciparum*. Rarely, *P. vivax* or *P. ovale* produce serious complications, debilitating relapses, and even death. WHO established criteria for severe malaria in order to assist future clinical and epidemiological studies (World Health Organization: Severe and

complicated malaria). In 2000, the WHO revised these criteria to include other clinical manifestations and laboratory values that portend a poor prognosis based on clinical experience in semi-immune.

Indicators of severe malaria and poor prognosis (WHO)

Manifestation	Features
Initial World Health Organization criteria from 1990	
Cerebral malaria	Unrousable coma not attributable to any other cause, with a Glasgow Coma Scale score ≤ 9. Coma should persist for at least 30 min after a generalized convulsion
Severe anemia	Hematocrit <15% or hemoglobin < 50 g/l in the presence of parasite count >10 000/µl
Renal failure	Urine output <400 ml/24 hours in adults (<12 ml/kg/24 hours in children) and a serum creatinine>265 µmol/l (> 3.0 mg/dl) despite adequate volume repletion
Pulmonary edema and acute respiratory distress syndrome	The acute lung injury score is calculated on the basis of radiographic densities, severity of hypoxemia, and positive end-expiratory pressure
Hypoglycemia	Whole blood glucose concentration <2.2 mmol/l (<40 mg/dl)
Circulatory collapse (algid malaria)	Systolic blood pressure <70 mmHg in patients > 5 years of age (< 50 mmHg in children aged 1–5 years), with cold clammy skin or a core-skin temperature difference >10°C
Abnormal bleeding and/or disseminated intravascular coagulation	Spontaneous bleeding from gums, nose, gastrointestinal tract, or laboratory evidence of disseminated intravascular coagulation
Repeated generalized convulsions	≥ 3 convulsions observed within 24 hours
Acidemia/acidosis	Arterial pH <7.25 or acidosis (plasma bicarbonate <15 mmol/l)
Macroscopic hemoglobinuria	Hemolysis not secondary to glucose-6-phosphate dehydrogenase deficiency
Added World Health Organization criteria from 2000	
Impaired consciousness	Rousable mental condition
Prostration or weakness	
Hyperparasitemia	> 5% parasitized erythrocytes or > 250 000 parasites/µl (in nonimmune individuals)
Hyperpyrexia	Core body temperature >40°C
Hyperbilirubinemia	Total bilirubin >43 µmol/l (> 2.5 mg/dl)

The major complications of severe malaria include cerebral malaria, pulmonary edema, acute renal failure, severe anemia, and/or bleeding. Acidosis and hypoglycemia are the most common metabolic complications. Any of these complications can develop rapidly and progress to death within hours or days. In many patients, several of these complications exist together or evolve in rapid succession within a few hours. In clinical practice, patients must be assessed for any of these signs or symptoms that suggest an increased risk for developing complications and must be treated immediately. In various studies risk factors for severe malaria and death include age greater than 65 years, female sex (especially when associated with pregnancy), nonimmune status, coexisting medical conditions, no antimalarial prophylaxis, delay in treatment, and severity of the illness at admission coma, acute renal failure, shock, pulmonary edema, coagulation disorders (Bruneel et al., 2003; Schwartz et al., 2001). In tropical countries with a high transmission of malaria (hyperendemic areas), severe malaria is predominantly a disease of young children (1 month to 5 years of age). In industrialized countries, most life-threatening complications occur in nonimmune travelers returning from endemic areas. Severe malaria accounts for approximately 5% of imported malaria cases (range 1–38%). The case fatality rate in returning travelers with falciparum malaria varies from 0.6% to 3.8%, and for severe malaria it may exceed 20%, even when managed in intensive care units (Kain et al., 1998).

Thrombocytopenia in *falciparum* malaria

Thrombocytopenia is the most common laboratory abnormality (60% of cases); followed by hyperbilirubinemia 40%, anemia 30%, and elevated hepatic aminotransferase levels 25% (D'Acremont et al., 2002). At the turn of 19th century malaria was thought to be associated with increase platelets count. However, in 1924 reduction in peripheral platelets concentration was described in man. Thrombocytopenia is a frequent finding in acute *falciparum* malaria infection. It results from a combination of platelet activation, splenic pooling and reduced life span due to activation of the intrinsic pathway. Platelets themselves or mediators derived from platelet and coagulation cascade may affect the interaction between infected erythrocyte and the endothelium and they are important in the pathophysiology of severe malaria. When platelets are activated they changed shape,

aggregate and release factors from their granule which stimulate a cascade of events resulting in platelets aggregation and adhesion to damaged endothelium and activate the coagulation pathway and also involved in the repair of endothelium and adjoining tissue.

Although infection of red cells is main feature of malaria, the key pathological event in the development of severe falciparum malaria is interaction between the infected cells and the microvascular endothelium where platelets and coagulation factors are important components. Profound thrombocytopenia is particularly associated with severe falciparum infection. In the past platelet and coagulation factors are considered to be a key importance in the pathogenesis of severe malaria through disseminated intravascular coagulation (DIC). But subsequent studies demonstrated that DIC and lethal hemorrhage are very rare. The definition of thrombocytopenia $<150\times10^9$L is conventionally applied to Caucasian, and it may not be applied in the tropical population, where a more appropriate cutoff may be $<100\times10^9$L (Malaria: a hematological perspective By Saad H. Abdalla, Geoffrey Pasvol). But in recent study of 150 adults who hospitalized with falciparum malaria in Thai-Burma border, the medium (range) admission platelets count was 41(2-254) 10^9L and 92% has $<150\times10^9$L.

Thrombocytopenia has often been encountered in infection with *Plasmodium falciparum*, especially when the organisms are resistant to chloroquine (Dennis et al., 1966). It has been suggested that platelet consumption, as part of a disseminated intravascular coagulation, is a possible mechanism, although this is unlikely to be the basis of thrombocytopenia in all cases. It has been shown; using platelets labeled with chromium-S1, that production of platelets is normal or increased during the development of thrombocytopenia associated with malaria. Bone marrow failure is therefore unlikely to be responsible for the development of thrombocytopenia (Beale et al., 1976). Sequestration and destruction of platelets in the spleen is another possible mechanism of thrombocytopenia, in conjunction with splenomegaly. Excessive removal of platelets, whether normal or immunologically changed by a hypertrophied reticuloendothelial system has been postulated. Electron microscopic confirmation of intraplatelet parasitism suggests that this is a possible cause of the decreased lifespan of platelets.

The introduction of techniques to study platelet kinetics has enabled the mechanisms of thrombocytopenia in disease states to be better defined (Aas et al., 1958). After injection of labeled platelets into normal subjects the percentage of the platelets recovered varies inversely with the size of the splenic pool and is a reliable indication of its size. In one study platelet recovery values after the injection of labeled platelets were greatly reduced. These recoveries are reported in subjects with congestive splenomegaly (Aster et al., 1966). And platelet pooling in the spleen appears to be a major factor in the etiology of the thrombocytopenia in malaria. Splenomegaly with congestion of the red cords is a characteristic feature of malaria and the increased pooling may simply be a reflection of this. However, in thrombocytopenic states due solely to excessive splenic pooling platelet life span is usually normal or near normal (Aster el al., 1966). Decrease in platelet life span could result from the removal of normal platelets by an abnormal reticuloendothelial system, or the removal of damaged platelets by a normal reticuloendothelial system, or a combination of both. Hyperplasia of reticuloendothial elements with evidence of increased phagocytic function is characteristic of the histology of the spleen and liver in malaria. One study assesses reticuloendothelial system phagocytic function in malaria by measuring the rate of clearance of ^{125}I-labelled human serum albumin (Sheugren et al., 1970). Phagocytic activity was markedly enhanced during the acute illness, returning to normal after treatment and complete recovery. The authors thought that this increased reticuloendothelial system activity may result in phagocytosis of normal platelets thus contributing to the thrombocytopenia in the disease. There is a rise in IgA, IgG, and IgM immunoglobulins primarily during the early period after infection (Collin et al., 1971). They suggested that in malaria immunologically altered platelets are removed at an excessive rate from the blood by the reticuloendothelial system. One study suggest that this role is a relatively minor one, as the platelet kinetics in the malaria patient do not resemble closely those reported in acute immune thrombocytopenia where platelet recovery is usually normal, platelet life span is reduced to a few hours or less, and platelet turnover is increased from 4 to 9 times (Aster et al., 1958). The results of these studies with ^{51}Cr-labelled platelets thus show at least two factors in the mechanism of the thrombocytopenia in malaria, excessive splenic pooling of platelets and decreased

platelet life span. The former mechanism is the more important, for the recovery values were extremely low while the decrease in platelet life span was moderate and not as severe as is usually found in patients with acute immune thrombocytopenia. Thrombocytopenia in severe malaria may be the result of D.I.C (Dennis et al., 1966). But D.I.C. is not a feature of uncomplicated (Shuiman et al., 1970). In one study coagulation tests failed to produce evidence of D.I.C in five of their patients. In one patient plasma fibrinogen was moderately decreased, but levels of F.R. antigen were only slightly raised. All patients in that study showed abnormally rapid removal of injected ^{125}I fibrinogen from the plasma. While the clearance rates were not as rapid as is usually found in D.I.C. they were nonetheless more rapid than those of the control subjects. This was not associated with changes in coagulation tests in five patients. In one patient with moderate reduction in plasma fibrinogen the rate of catabolism of ^{125}I fibrinogen was not more rapid than the group as a whole. Thus no supportive evidence has been found that the rapid removal of ^{125}I fibrinogen was associated with D.I.C. Of particular importance is the finding of little or no rise in F.R. antigen levels. Fibrinogen is catabolized directly, without preliminary conversion to fibrin, so that fibrinogen catabolism can be changed without involving coagulation (Regoeczi et al., 1970). The site of fibrinogen catabolism is probably the cells of reticuloendothelial system, for experimentally induced hyperendocytosis of the reticuloendothelial system is accompanied by an increased rate of fibrinogen catabolism in rabbits (Regoeczi et al., 1970). It is possible that in uncomplicated malaria rapid disappearance from the plasma of injected ^{125}I fibrinogen reflects clearance into the overactive reticuloendothelial system; a mechanism similar to that found with ^{125}I-labelled microaggregated human serum albumin (Sheugren et al., 1970). However, when the clearance of injected ^{125}I fibrinogen from the plasma is very rapid in malaria patients this may be on the basis of D.I.C. Evidence for D.I.C. in some patients with malaria is irrefutable. Results of one study, however, indicate that this is not the only mechanism which may produce thrombocytopenia in malaria. The clinical implications of these findings relate particularly to the use of heparin in malaria. In the presence of unequivocal D.I.C. the use of heparin is justified in malaria. The results of one study, however, indicate that if heparin is to be used

evidence of D.I.C. other than thrombocytopenia and increased fibrinogen catabolism must be found, particularly in uncomplicated cases.

Another mechanism for the production of thrombocytopenia was postulated by Devakul and Dennis (Dennis et al., 1966; Devakul et al., 1966). They felt that the platelets were being removed from the circulation by consumption in intravascular coagulation, and presented convincing evidence by showing depletion of coagulation factors and the presence of fibrinogen degradation products as well as thrombocytopenia. But Dennis and Conrad's studies were for the most part carried out on experimental malaria in monkeys or on humans with a late relapse of drug-resistant *P.falciparum* malaria (Devakul et al., 1966). One study also found a pronounced drop in fibrinogen concentration in two out of six cases of *falciparum* malaria. But one study could not find depletion of clotting factors in their studies of humans with *P. falciparum*, *P. vivax*, or *P. ovale* infections or of monkeys with *P. cynomolgi* or *P. knowlesi* infections, despite the presence of thrombocytopenia (Shuiman et al., 1970). One study found no significant abnormalities in the prothrombin times or in the fibrinogen levels. However, thrombocytopenia can develop independently of intravascular coagulation and that, in malaria, the demonstration of thrombocytopenia alone cannot be used as a basis for diagnosing intravascular coagulation. It is therefore important to show depletion of clotting factors before making the diagnosis, and before giving the specific but potentially dangerous antidote, heparin. Removal of presumably normal platelets in malaria by a hypertrophied reticuloendothelial system is supported by the frequent finding of an enlarged spleen and liver, and morphologically there is evidence for the involvement of macrophages in the elimination of parasites, pigment, and erythrocytes. No endotoxin has ever been found to aid the removal of platelets from the circulation, but antimalarial antibodies have been found sufficiently early in the illness to be considered an influence in platelet destruction. One study measure the immunoglobulins, particularly the IgM fraction, the rise of which is known to tally closely with the formation of malarial antibody (Tobbie et al., 1966; Zuckerman et al., 1969). They carefully emphasized that all gammaglobulin is not antibody, and others studies also pointed out that raised levels of immunoglobulins can be due to intercurrent infections. As the thrombocytopenia was most profound at this early

stage, between Day 0 and Day +4 of treatment, malarial antibody has to be considered as a factor in the production of the thrombocytopenia. In one study the level of immunoglobulin IgM seemed to be falling by the twenty-eighth day (Abele et al., 1965). One study found the IgM fraction (or its equivalent 19s macroglobulin) to be increased towards the beginning of the illness, and that as the IgM level began to fall the immunoglobulin IgG (or its equivalent 7s gammaglobulin) began to rise and remained high as a more permanent indication of previous malarial infection. Quinine has been incriminated as a factor in the pathogenesis of blackwater fever in falciparum malaria, and, not unnaturally, both quinine and other antimalarial drugs have been scrutinized for undesirable haematological side effects. But one study concluded that drugs played no part in the pathogenesis of thrombocytopenia (Beale et al., 1970).

Low platelet counts have been consistently found for both *P. falciparum* and *P. vivax* (Rajanasthien et al., 1992). Platelet counts less than 100,000/L. *Plasmodium falciparum* cases with platelet levels below this count had been documented in other populations of semi immune *and* non-immune patients. The trend of decreasing platelet count with increasing levels of parasitemia has been previously noted for *P. falciparum*. Decreased platelet production has been ruled out (Perin et al., 1982). Thrombocytopenia is a result of peripheral destruction and consumption. Immune complexes generated by malarial antigen lead to sequestration of the injured platelets by macrophages in the spleen (Skudowitz et al., 1972). Platelet consumption in disseminated intravascular coagulation contributes to thrombocytopenia in complicated *P. falciparum* malaria. Platelet dysfunction resulting in hyper aggregation is another alteration occurring in association with malaria resulting in hyper aggregation (Mohanty et al., 1988). During malaria infection, there are several factors that activate platelets, among which are formation of immune complexes and damage to endothelial cells. Surface contact of platelet membrane to with parasitized RBCs is another stimulator (Inyang et al., 1987). Intravascular lysis of the activated platelets may also occur (Essien et al., 1989). Trends between increasing parasite density with a decrease in the level of hematologic parameters other than platelet count were not having been noted in the literature.

One study has showed that thrombocytopenia in children with acute malaria is strongly associated with plasma concentrations of IL-10 but not with *P. falciparum* parasitemia or other plasma cytokines. There is evidence that IL-10 may directly induce thrombocytopenia. The administration of a low dose of recombinant human IL-10 (8 g/kg/d) decreased platelet production in healthy adult volunteers (Sasman et al., 2000). In the same study, there was a corresponding reduction in splenic sequestration of platelets in the IL-10–treated group compared with the placebo treated subjects. In the IL-10–treated group, there was a trend toward lower numbers of megakaryocyte colony-forming units (CFU-MKs) compared with volunteers who received placebo. This single study suggests that IL-10–induced reduction in platelet count is caused, at least in part, by a reduction in platelet production. The clinical relevance of thrombocytopenia associated to *P. falciparum* malaria has been addressed in two large studies. In a clinical study in Kenyan children, platelet counts $< 150 \times 10^9$/L were found in 57% of children with acute malaria and were associated with age, prostration, and parasite density, but not with bleeding problems or mortality (Ladhni et al., 2002). On the other hand, a recent study carried out in a hypoendemic area in Senegal identified thrombocytopenia as an independent predictor of death (odds ratio = 13.3). However, other data from Southeast Asia do not support a relationship between thrombocytopenia and outcome (Newton et al., 2004). These inconsistent relationships between disease severity or outcome and thrombocytopenia may reflect the differences in clinical epidemiology and the corresponding differences immune status and immunopathology in patients with severe malaria Indeed, the association between thrombocytopenia and IL-10 levels in malaria infection may explain, at least in part, the failure to find consistent associations of thrombocytopenia and poor outcome in malaria infection despite the evidence from clinical experimental and animal studies for a role of platelets in the pathophysiology of malaria. IL-10 itself is associated with less severe forms of clinical malaria (Kurtzhals et al., 1998). And this relationship would confound any simple relationship between thrombocytopenia and outcome. It is possible that a reduction in platelet count mediated by IL-10 reduces the pathologic interaction between infected erythrocytes and platelets, associated with severe disease and is

therefore one mechanism whereby IL-10 improves the outcome of severe malaria (Pain et al., 2001).

One study demonstrated that vWF and vWF propeptide secretion were significantly increased at a very early stage in *P. falciparum* blood-stage infection, pointing at acute endothelial cell activation in malaria. Increased amounts of activated vWF, exposing the gpIba-binding site of vWF for platelets, were observed as well. They find that activated vWF may therefore be an important inducer of thrombocytopenia during early malaria and may as such contribute to the pathogenesis of malaria. They are the first to demonstrate increased levels of activated vWF in an infectious disease. The findings of a very early decrease in platelet number, together with elevated levels of activated vWF, suggest that early intravascular platelet adhesion and clumping may also be a prominent and unique feature of malaria. Circumstantial evidence for this was provided by an autopsy study from Malawi that showed increased platelet accumulation in the brains of children who died of cerebral malaria, compared with that in children who died of nonmalarial encephalopathy. In addition, electron microscopy studies have also demonstrated platelet adherence to brain endothelial cells during both human and murine cerebral malaria. Besides causing thrombocytopenia, vWF-mediated platelet clumping may contribute to the pathogenesis of malaria in several ways. First, adhering and aggregating platelets may cause organ perfusion failure and tissue hypoxia. Second, vWF may play a role in the cytoadherence of parasitized red blood cells to vascular endothelium during the early stages of malaria. Multiple endothelial receptors are involved in this process, most notably CD36 and intercellular adhesion molecule–1. However, up-regulation of these endothelial receptors takes time, because they require de novo synthesis. In contrast, stored activated vWF can immediately be secreted from endothelial cells with subsequent binding of platelets. It has recently been demonstrated that platelets may facilitate cytoadherence by acting as bridges between parasitized red blood cells and endothelial cells. Last, vWF-mediated platelet adhesion may be important in brain microvessels that express only a little CD36. Platelets expressing high levels of CD36 could deliver the required CD36 in these situations. There were also 3 cross-sectional studies that have previously analyzed vWF concentrations in patients with malaria, and all found elevated levels. In a report

the inverse relationship between vWF levels and platelet numbers was demonstrated. The authors concluded that thrombocytopenia during malaria is not indicative of disseminated intravascular coagulation but may relate to endothelial damage. In the other 2 studies, the relationship between vWF and platelet number was not reported. That vWF-mediated platelet clumping plays an important role in the development of thrombocytopenia and in the pathogenesis of malaria. The experimental human malarial infection model, however, enables comparison of the kinetics of these variables during the very early stages of malaria. This allows a more reliable assessment of the direct effect of *P. falciparum* on endothelial cells and platelets. In addition, several other pathogenic mechanisms for malaria-induced thrombocytopenia during more advanced stages of malaria have been suggested, such as splenic sequestration, oxidative stress, platelet apoptosis, and antibody- and cell-mediated immunity. Activation of the coagulation system may also cause platelet consumption. However, the coagulation is usually only mildly activated during malaria, and disseminated intravascular coagulation is rare, even during severe malaria, because both vWF and vWF propeptide are predominantly synthesized in vascular endothelial cells and are released on endothelial cell activation. Endothelial cell pathophysiology is complex and that changes may encompass a spectrum ranging from simple perturbation to activation and even endothelial cell damage. Early endothelial cell activation may be critical during the early stages of malaria. Sequestration of parasitized red blood cells containing the more mature stages of the malarial parasite is important for parasite survival. In one study they demonstrated that the mean asexual life cycle of malarial parasites is 43.7 h and have provided evidence that parasitized red blood cells indeed adhere to vascular endothelium within 2 days after release from the liver. In this way, early removal and destruction by the spleen is avoided. Early endothelial cell activation probably facilitates this sequestration by, for example, up-regulating endothelial adhesion molecules and vWF release. Inflammatory cytokines are known endothelium agonists and have been shown to directly stimulate the release of vWF from endothelial cells or to inhibit ADAMTS13. In the past, levels of proinflammatory cytokines started to increase 1–2 days before treatment initiation during experimental malaria. Alternative mechanisms may also be involved in the initiation of endothelial cell activation and vWF release. A still-

undetermined factor released at the end of liver schizogony or, alternatively, malarial parasite products could be potential candidates. One of these parasite products, glycosylphosphatidylinositol anchors, has already been linked to endothelial cells by its potential to induce cytokine production and up-regulate the expression of endothelial adhesion molecules directly. *P. falciparum* parasitized red blood cells also have the capacity to directly stimulate human endothelial cells. However, it is important to realize that, at the time when levels of vWF and vWF propeptide started to rise, only a very small percentage of erythrocytes (estimated at 0.001%) were infected and that malarial antigen levels, including of glycosylphosphatidylinositol, were probably still low. Plasma concentrations of vWF are influenced by blood group. Subjects with blood group O have □30% lower levels of vWF than do those with blood group A, B, or AB. Subjects with blood group O are relatively resistant against severe malaria. In one study which included 14 volunteers, there was no significant difference in median vWF levels between the 7 volunteers with blood group O and the 7 with blood group non-O. They concluded that *P. falciparum* induces systemic acute endothelial cell activation and release of activated vWF immediately after the onset of the blood stage of the infection. The phenomenon may be responsible for the early thrombocytopenia and may play a role in the pathogenesis of malaria by providing a link between parasitized red blood cells and endothelial cells.

The leukocyte count is usually normal or low, but neutrophilia with a marked increase in band forms (left shift) is present in the majority of cases. The erythrocyte sedimentation rate, C-reactive protein, and procalcitonin are almost invariably elevated. The severity of malaria corresponds to the degree of the laboratory abnormalities. In one study of travelers who returned from the tropics, thrombocytopenia and hyperbilirubinemia had a positive predictive value of 95% for malaria.

Light microscopy of thick and thin stained blood smears remains the standard method for diagnosing malaria. Thick smears are 20–40 times more sensitive than thin smears for screening of *Plasmodium* parasites, with a detection limit of 10–50 trophozoites/μl. Thin smears allow one to identify malaria species (including the diagnosis of mixed infections), quantify parasitemia, and assess for the presence of schizonts, gametocytes, and malarial pigment in neu-trophils and monocytes. The

diagnostic accuracy relies on the quality of the blood smear and experience of laboratory personnel. Before reporting a negative result, at least 200 oil immersion visual fields at a magnification of 1000× should be examined on both thick and thin smears, which have a sensitivity of 90%. The level of parasitemia may be expressed either as a percentage of parasitized erythrocytes or as the number of parasites per microliter of blood. In non *falciparum* malaria, parasitemia rarely exceeds 2%, whereas it can be considerably higher (>50%) in falciparum malaria. In nonimmune individuals, hyperparasitemia (>5% parasitemia or >250000 parasites/μl) is generally associated with severe disease. In falciparum malaria, parasitized erythrocytes may be sequestered in tissue capillaries resulting in a falsely low parasite count in the peripheral blood ('visible' parasitemia). (Manson's *Tropical Diseases*). In such instances, the developmental stages of the parasite seen on blood smear may help to assess disease severity better than parasite count alone. The presence of more mature parasite forms (>20% of parasites as late trophozoites and schizonts) and of more than 5% of neutrophils containing malarial pigment indicates more advanced disease and a worse prognosis (Nguyen et al., 1995). One negative blood smear makes the diagnosis of malaria very unlikely (especially the severe form); however, smears should be repeated every 6–12 hours for 48 hours if malaria is still suspected.

Although examination of the thick and thin blood smear is the 'gold standard' for diagnosing malaria, important advances have been made in diagnostic testing, including fluorescence microscopy of parasite nuclei stained with acridine orange, rapid dipstick immunoassay, and polymerase chain reaction assays. Sensitivity and specificity of some of these methods approach or even exceed those of the thin and thick smear (Lee et al., 2002). Rapid dipstick immunoassays detect species-specific circulating parasite antigens targeting either the histidine-rich protein-2 of *P. falciparum* or a parasite-specific lactate dehydrogenase. Although the dipstick tests may enhance diagnostic speed, microscopic examination remains mandatory in patients with suspected malaria, because occasionally these dipstick tests are negative in patients with high parasitemia, and their sensitivity below 100 parasites/μl is low (Moody et al., 2002). Tests based on polymerase chain reaction for species-specific Plasmodium genome are more sensitive and specific than are other tests, detecting as

few as 10 parasites/µl blood.. Antibody detection has no value in the diagnosis of acute malaria. It is mainly used for epidemiologic studies.

The treatment of malaria is depends on (1) the infecting species of plasmodium (2) severity of the infection and (3) geographical area and drug resistance. *Falciparum* malaria in patients who are non immune (never have malaria infection before) who living in malaria free area is a medical emergency and require repaid initiation of anti malaria therapy. If the species cannot be identified immediately, the patients should be assumed to have drug resistance *falciparum* malaria until prove otherwise. Uncomplicated *falciparum* malaria may be treated with oral therapy whereas for severe type, it needs parenteral therapy.

Intravenous quinine is currently the most widely used agent in the treatment of severe falciparum malaria, usually formulated as a dihydrochloride salt. The drug acts by inhibiting the hemosoin biocrystlliztion, thus facilitating an aggregation of cytotoxic heme. Toxic free heme accumulates in the parasites, leading to their death. The adverse effect of quinine may cause cinchonism (bitter taste, dysphoria, tremor, tinnitus, reversible high-tone hearing loss, headache, nausea, vomiting, and abdominal pain) or pruritus, which should not lead to dose reduction. Severe toxicities include cardiac arrhythmias, hypotension, blindness, deafness, and hyper-insulinemic hypoglycemia.

The earliest recorded historical account of quinine comes from 1633 when an Augustinian monk in Lima, Peru wrote about a powder of cinchona “given as a beverage to cure the fevers.” According to legend, in 1638 the Jesuits used quina bark to cure the Countess of Cinchón, wife of the Viceroy of Peru, of her fevers. Both the Countess and the Jesuits then brought this Peruvian bark to Europe. It was called “Jesuit powder” or “Countess’s powder”.

It was an English apothecary’s assistant named Robert Talbor who, in the mid-1600s, realized that the fevers responsive to cinchona bark were those specifically associated with malaria. After Talbor’s death in 1681, the King of France disclosed the secret ingredient. In 1820, Pierre-Joseph Pelletier and Joseph Bienaimé Caventou isolated an alkaloid from cinchona (or quina) bark and named it quinine. The purified compound began to be used instead of powdered bark to treat malaria. Subsequent experiments established that this compound is the most active antimalarial constituent

of cinchona bark. It wasn't until 1854 that the structure of quinine was elucidated. By the late 1800s, the malaria parasite had been identified and Ronald Ross had discovered the role of the mosquito vector. Ross launched "mosquito brigades" to eradicate the vector in England, while another public health advocate, S. P. James, advocated improving housing to separate humans from mosquitoes.

Efforts to synthesize quinine in the laboratory had been underway since 1850, when the French Society of Pharmacy urged chemists to produce synthetic quinine. It took 50 years to progress from the identification of quinine's empirical formula to an understanding of how the atoms are arranged in the molecule, and it took another 40 years before stereochemical problems could be worked out. Hoffmann-La Roche disclosed the first total synthesis of quinine in 1970, although stereocontrol was still incomplete. Gilbert Stork of Columbia University achieved fully stereochemically controlled total synthesis of quinine in 2001.

Artemisinin (Qinghaosu) is and endoperoxide containing compound extracted from the leaves of a plant called Artemisia annua (sweet wormwood). It is isolated by chinese scientists in 1972. Its antimalarial application was first described in Zhouhou Beji Fang ("The Handbook of Prescriptions for Emergencies"), Artemisinin take critical role in treatment of malria because of high activity and low resistance repaid onset of action and repaid clearance rate. Artemisinin is a rapid parasiticidal of the asexual stages; it is anti-gametocyte and blocks sporogony. It produces ultra-structural changes to the growing trophozoite parasite. A whorl is produced in the food vacuole and the parasite's mitochondria proliferated. This reduces parasite's survival. Endoperoxide bridge is essential for its anti-malarial activity. The compound is activated by the intra-parasitic haem to irreversibly decompose, generating free radicals that alkylate and oxidises proteins and lipids. The membrane of the parasite is damaged by lipid peroxidation and channel proteins' inactivation. The World Health Organisation has recommended that a switch to Artemisinin based combination therapy should be made in all countries where the malaria parasite has developed resistance to chloroquine. The mechanism of Artemisinin to killing of malaria parasite is mediated by production of free radicals. Artemisinin derivatives lacking endoperoxide bridge are lacked of anti-malaria activity. By addition of free radical generation compounds enhances anti-malarial activity. Artemisinin derived free

radical bind to protein through alkylation. Artemisinin inhibit hemozoin biosynthesis and inhibit hemoglobin digestion by malaria parasites and form covalent adduct with malaria parasites. Currently there is no evidence of Artemisinin resistance because of it short half-life, reduces transmission rates and used as combination with other anti-malarial drugs. There are numbers of analogues and derivative in Artemisinin famaliy they are Artesunate, Artemeter, Artether and Dihydoartimisinin. Artemeter is available for intramsucular injection as well as Artesunate, it has water soluble property and it is haemisuccinate estar and can be administered intravenously or intramuscularly in solution as artesunic acid, or orally as 600 mg. Arteminisin and artemether suppositories are available commercially. Adverse effects are very rare except neurotoxicities in animals with very high dose.

Parenteral chloroquine is the drug of choice for severe chloroquine-susceptible *P. falciparum* infections (originating from Central America north of the Panama Canal, Haiti, Dominican Republic, Argentina, Paraguay, Egypt, Syria, Turkey, Saudi Arabia, Iraq, Azerbaijan, and Mauritius) and for those rare cases of life-threatening malaria caused by *P. ovale*, *P. malariae*, and *P. vivax* (except for infections from Papua New Guinea, Sumatra, Irian Jaya, Myanmar, Vanuatu, India, and the Amazon region of Brazil). It is highly effective against erythrocytic forms of *P. vivax*, *P. ovale* and *P. malariae*, sensitive strains of *P. falciparum* and gametocytes of *P. vivax*. It rapidly controls acute attack of malaria with most patients becoming afebrile within 24-48 hours. It is more effective and safer than quinine for sensitive cases.

Chloroquine was discovered by a German, Hans Andersag, in 1934 at Bayer I.G. Farbenindustrie A.G. laboratories in Eberfeld, Germany. He named his compound resochin. Through a series of lapses and confusion brought about during the war, chloroquine was finally recognized and established as an effective and safe antimalarial in 1946 by British and U.S. scientists. Chloroquine may have a more rapid effect on lowering parasitemia than either quinine or quinidine, but it also has a more profound hypotensive side effect. Chloroquine should be given by a controlled intravenous infusion with a loading dose 10 mg/kg base over 8 hours, followed immediately by a maintenance dose of 15 mg/kg base infused over 24 hours. The mechanism of action of chloroquine is unclear. Being alkaline, the drug reaches high concentration within the food vacuoles of the parasite and raises its pH. It is found to

induce rapid clumping of the pigment. Chloroquine inhibits the parasitic enzyme heme polymerase that converts the toxic heme into non-toxic hemazoin, thereby resulting in the accumulation of toxic heme within the parasite. It may also interfere with the biosynthesis of nucleic acids. Other mechanisms suggested include formation of drug-heme complex, intercalation of the drug with the parasitic DNA etc.

Chloroquine is a relatively safer anti malarial. At therapeutic doses, it can cause dizziness, headache, diplopia, disturbed visual accomodation, dysphagia, nausea, malaise, and pruritus of palms, soles and scalp. It can also cause visual hallucinations, confusion, and occasionally frank psychosis. These side effects do not warrant stoppage of treatment. It can exacerbate epilepsy. When used as prophylactic at 300 mg of the base/ week, it can cause retinal toxicity after 3-6 years (i.e. after 50-100 g of chloroquine). Intra muscular injections of chloroquine can cause hypotension and cardiac arrest, particularly in children.

Primaquine (PRIM-a-kween) belongs to the group of medicines called antiprotozoals. It is used in the treatment of malaria. Primaquine is the essential co-drug with chloroquine in treating all cases of malaria. It is highly effective against the gametocytes of all plasmodia and thereby prevents spread of the disease to the mosquito from the patient. It is also effective against the dormant tissue forms of *P. vivax* and *P. ovale* malaria, and thereby offers radical cure and prevents relapses. It has insignificant activity against the asexual blood forms of the parasite and therefore it is always used in conjunction with a blood schizonticide and never as a single agent. Mechanism of action is not well understood. It may be acting by generating reactive oxygen species or by interfering with the electron transport in the parasite. In therapeutic doses, primaquine is well tolerated. At larger doses, it may cause occasional epigastric distress and abdominal cramps. This can be minimised by taking the drug with a meal. Mild anemia, cyanosis and methemoglobinemia may also occur. Severe methemoglobinemia can occur rarely in patients with deficiency of NADH methemoglobin reductase. Granulocytopenia and agranulocytosis are rare complications.

Patients with deficiency of Glucose 6-phosphate dehydrogenase will develop hemolytic anemia on taking usual doses of primaquine. This problem is restricted to certain sections of the population. The drug should be stopped when signs of

hemolysis and anemia are observed. It may not be practical to test each and every patient for G 6 PD deficiency before administering primaquine. Primaquine should not be used in patients who have severe systemic illness that is likely to cause leukopenia (severe rheumatoid arthritis, SLE etc.). It should not be used with other drugs likely to cause bone marrow depression. Patients with G6PD deficiency may develop hemolysis with quinine. A study in healthy subjects indicates that concurrent administration of primaquine can increase blood concentrations of mefloquine and may increase the adverse effects due to mefloquine. Therefore, simultaneous use of quinine or mefloquine is contra indicated.

Mefloquine was born during the Vietnam war, as a result of research into newer anti malarials, to protect the American soldiers from the multi drug resistant falciparum malaria. Nothing much has happened after that and hence this 'new' drug should be restricted for use against multi drug resistant falciparum only. Mefloquine has been found to produce swelling of the *P. falciparum* food vacuoles. It may act by forming toxic complexes with free heme that damage membranes and interact with other plasmodial components. It is effective against the blood forms of falciparum malaria, including the chloroquine resistant types. It is generally well tolerated in therapeutic doses up to 1500 mg. Nausea, vomiting, abdominal pain and dizziness can occur in doses exceeding 1 g. Less frequently it can cause nightmares, sleeping disturbances, dizziness, ataxia, sinus bradycardia, sinus arrhythmia, postural hypotension, and an 'acute brain syndrome' consisting of fatigue, asthenia, seizures and psychosis. Mefloquine should be used with caution in patients with heart block, patients taking beta blockers, patients with history of epilepsy and psychiatric disease. It should be avoided in first trimester of pregnancy and pregnancy should be avoided within 3 months of taking the drug.

Recommended regimens for initial parenteral treatment of severe falciparum malaria

Drug	Loading dose[1]	Maintenance dose	Comments
Regimen 1			
Quinine dihydrochloride salt (available outside the USA), reconstituted in 5% glucose or normal saline	7 mg salt/kg iv over 30 min followed immediately by maintenance dose OR20 mg salt/kg over 4 hours, followed 8 hours later by maintenance dose	10 mg salt/kg diluted in10 ml/kg isotonic fluid iv over4 hours repeated every 8 hours[2]	If hemodialysis is performed, then quinine is administered after dialysis. Monitor blood glucose because of risk for developing hyperinsulinemic hypoglycemia
PLUS (either concurrently or immediately thereafter)			
Doxycycline[3]	Not required	1.5 mg/kg (usually 100 mg) po or iv every 12 hours for 7 days	Should not be given to pregnant or breast-feeding women or children < 8 years old
Regimen 2			
*Quinidine gluconate(*available in the USA), reconstituted in normal saline	10 mg salt/kg (equivalent to6.2 mg base/kg) iv infused over 1–2 hours, followed immediately by maintenance dose	0.02 mg/kg/min salt (equivalent to 0.0125 mg/kg/min base) continuous iv infusion[2]	Electrocardiographic monitoring is mandatory; slow or stop infusion if QRS lengthens >25% of baseline value or QTc interval > 500 ms
PLUS (either concurrently or immediately thereafter)			
Doxycycline[3]	Not required	Same as above	

Regimen 3

Artesunate	2.4 mg/kg iv bolus	1.2 mg/kg iv daily[4]	Artesunic acid 60 mg is dissolved in 0.6 ml 5% sodium bicarbonate, diluted to 3–5 ml 5% glucose, and given immediately by iv bolus injection
PLUS			
Mefloquine	15 mg/kg (750 mg) base	10 mg/kg (500 mg) base po at 6–8 hours and (if >60 kg) followed by 5 mg/kg (250 mg) po at 16 hours	Total dose: 1500 mg

Regimen 4

Artemether	3.2 mg/kg im	1.6 mg/kg im daily[4]
PLUS		
Mefloquine	Same as above	Same as above

Loading dose should not be administered to patients who received quinine, quinidine, halofantrine, or mefloquine within the preceding 12 hours. [2]Intravenous quinine or quinidine should be given for at least 24 hours but oral antimalarial treatment should be substituted as soon as the patient is stable and can take oral therapy to complete the treatment course. If intravenous treatment is continued past 48 hours, then the maintenance dose should be reduced by 30–50%. In renal failure and in dialysis patients, the maintenance dose of quinine should be reduced by 30–50%. [3]Clindamycin 5 mg/kg (usually 300 mg) po or iv every 8 hours can be administered if the patient is unable to take doxycycline. [4]Parenteral artesunate or artemether should be given for at least 3 days but oral antimalarial treatment should be substituted as soon as the patient is stable and can take oral therapy to complete the treatment course. im = intramuscularly; iv = intravenously; po, orally.

Recommended oral treatment for severe falciparum malaria after initial parenteral therapy for at least 24 hours when clinical improvement is evident and the patient can tolerate oral medication

Drug	Dose	Comments
Artemether/lumefantrin	80 mg arthemeter/480 mg lumefantrin once daily for 3 days	Well tolerated, faster parasite clearance, but longer fever resolution time
Atovaquone/proguanil	1000 mg atovaquone/400 mg proguanil at 0, 8, 24, 36, 48 and 60 hours	Well tolerated, more effective than mefloquine in treatment of multidrug-resistant falciparum malaria
Mefloquine	15 mg/kg (750 mg) base at 0 hours, followed by10 mg/kg (500 mg) base at 6–8 hours, and (if >60 kg) followed by 5 mg/kg (250 mg) at 16 hours	Contraindicated in persons with seizure or psychiatric disorders, or with cardiac conduction abnormalities
Quinine (sulfate salt)	10 mg salt/kg (600–650 mg) every 8 hours to complete7 days of treatment (total duration)	Side effects include cinchonism and pruritus

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CHAPTER IV
MATERIALS AND METHODS

3.1 Place of Study, Study Design and Study Period

This retrospective study was carried out on patients both male and female who were admitted to Hospital for Tropical Diseases, Bangkok. The Hospital for Tropical Diseases is 160 beds hospital under the apices of Faculty of Tropical Medicine, Mahidol University. The hospital received mainly referred patients from surrounding providence and sub-urban area of the city where malaria is endemic and patients from Bangkok who have visited to malaria endemic area. This is special well reputed hospital for it patients management, well established modern investigation facilities and well known research activities for tropical diseases.

3.2 Inclusion criteria

(a) Patients with confirmed diagnosis of malaria by either (i) the finding of asexual forms of malaria parasites in the blood by light microscopy or (ii) a positive rapid diagnosis test, for the presence of malaria parasite antigen.
(b) All the participants must be older than 12 years and both genders can be accepted
(c) All the patients must have hospitalization at least 7 days.

3.3 Exclusion criteria

(a) Pregnant women and lactating mother are not suitable for this study
(b) Co-exiting infections (leptospirosis, scrub typhus, DHF etc)
(c) Clinical evidence of severe malnutrition or clinically significant disorders
(d) Blood transfusion given during the study period
(e) Evidence of *P.vivax*, *P.ovale*, *P.malariae* or mixed infections

3.4 Sample Size

Estimately and retrospectively, 220 patients were included in the study.

3.5 Materials and Methods

For retrospective data collection in this study we used the records of patient profiles in Hospital for Tropical Disease from 2005 to 2008. The patients admitted had fever and a positive blood smear of asexual form of *Plasmodium falciparum* malaria. The patients who received blood transfusion before the first sample of blood was taken would be excluded. The patients who have evidence of severe malnutrition or clinically significant disorders are also excluded from the study. Mixed infections are also excluded from the study.

After being admitted to the ward before any treatment was given, the patients underwent a complete history and full clinical examination. After assessment of the severity of the presentation, first sample of blood was collected for thin and thick blood smears and routine hematological investigations including complete blood count, hematocrit, white blood count and differential count, reticulocyte counts, platelet counts and parasite count, G6PD, hemoglobin type and Coomb's test. Biochemistry: liver function tests, electrolytes, blood sugar, blood urea nitrogen, creatinine, urinalysis and stool examination were done.

A definitive diagnosis of malaria is made by prompt microscopic examination of thick and thin blood films. Thick and thin blood films were stained by Gimsa's stain. Thick films contain many layers of RBCs, which lyses when stained, and are useful for screening of Malaria infection. A thin film is a monolayer that is fixed before staining so that the RBCs do not lyse; it is useful for quantifying infection and for determining the species of Plasmodium. We do not wait for a peak of fever before undertaking these investigations. By examination of the blood film it allows accurate speciation of the parasite and determination of parasite density. Occasionally, the initial blood film is negative, particularly when chemoprophylaxis has been taken, and the film was repeated when the clinical suspicion is high. The films were taken daily for 3 days (off antimalarial drugs). Body temperature was recorded every 4 hr. Fever clearance was assessed from the time the specific treatment was given until the body temperature become normal (37'C). Parasite clearance was assessed the same way, started from the time the specific treatment was given until the asexual form of *Plasmodium falciparum* was disappeared from the circulation.

A number of measures were applied to all patients with clinically diagnosed or suspected severe malaria. Antimalarial chemotherapy was given paraenterally (intravenously or intramuscularly). Oral treatment was substituted as soon as possible. It is also necessary to eliminate other possible causes of coma in the unconscious patient, so lumbar puncture was carried out. The patient was monitored for hypoglycemia, and glucose was given if necessary. The rate of infusion of I.V. fluids was carefully monitored, also urine production. A high body temperature was reduced by antipyretics or sponging.

The administration of a prophylactic anticonvulsant such as phenobarbital sodium (10-15 mg/kg body weight, intramuscularly) was advised in severe malaria. Regular monitoring is essential, and the patient was nursed on his/her side to avoid the risk of aspiration of fluid. The patient was not allowed to lie in a wet bed, and was advised to turn every couple of hours. The airways was kept open, and if rectal temperature (monitored every 4 hours for at least the first 48 hours after infection) rises above 39°C then paracetamol was given, and sponging initiated. Drugs which increase the risk of gastrointestinal bleeding such as aspirin or corticosteroids was avoided. (Management of severe and complicated malaria: a practical handbook. WHO, Geneva. 1991).

The drugs currently appropriate for the treatment of malaria vary according to the type of malaria. For chloroquine sensitive malaria, chloroquine was administered at 10mg base/kg of body weight in isotonic fluid by constant rate I.V. infusion over 8 hours followed by 15mg base/kg body weight was given over the next 24 hours. Alternatively, chloroquine 5mg base/kg body weight in isotonic fluid by constant rate IV infusion over 6 hours was given, repeated every 6 hours for a total of 5 doses. If IV infusion is not possible, oral chloroquine 3.5mg base/kg was given every 6 hours intramuscularly until the total dose is 25mg base/kg. Oral therapy was initiated as soon as the patient can swallow. (Management of severe and complicated malaria: a practical handbook. WHO, Geneva, 1991).

For chloroquine resistant malaria (largely diagnosed by the geographical area in which the malaria was contracted), a loading dose of 20mg quinine dihydrochloride salt/kg body weight was infused over 4 hours, in 5% dextrose saline. An infusion pump may be used to introduce 7mg salt/kg over 30 minutes, if available. Next a

maintenance dose of quinine 10mg salt/kg in dextrose saline was administered 12 hours after the loading dose (no delay is needed if an infusion pump was used). The maintenance dose was repeated every 8-12 hours until the patient can take oral therapy.

Alternatively, quinidine gluconate (only used if paraenteral quinine is unavailable) was infused as a loading dose of 15mg base/kg body weight over 4 hours. The maintenance dose was 7.5mg base/kg and repeated every 8 hours until oral medication can be taken. In all cases, loading doses are not required if the patient has taken quinine or quinidine or mefloquine in the preceding 7 days. If patients require more than 48 hours paraenteral therapy, then the maintenance doses of quinine and quinidine was reduced by one third to one half. (Management of severe and complicated malaria: a practical handbook. WHO, Geneva. 1991).

Oral therapy was depended on parasite sensitivity and drug availability. Quinine tablets, 10mg/kg, every 8 hours to complete 7 days treatment are a common treatment. Alternatively 15mg/kg mefloquine was given in 2 doses 12 hours apart was given (but not to pregnant women). A recent study suggests that higher than normal (i.e. 25 mg/kg as opposed to 15mg/kg) doses of mefloquine are considerably more effective - although unpleasant side effects such as dizziness, anorexia over the 7 days after treatment, and vomiting which may necessitate re-treatment, may be observed. Another useful oral antimalarial drug is halofantrine, 8mg base/kg every 6 hours for three doses, although this must not be given to pregnant women. Where there is significant quinine resistance (as in malaria contracted in Thailand, Cambodia and Vietnam) an oral dose of tetracyclines, 250mg four times a day, for seven days of treatment was given. It is dangerous to give IV tetracyclines, and was not given to pregnant women or children under 8 years of age (Management of severe and complicated malaria: a practical handbook, WHO).

All of the drugs mentioned above have certain problems associated with them. Quinine, the drug of choice for severe malaria, may cause serious hypoglycemia, and quinine poisoning is treated with oral activated charcoal. Chloroquine, still the most widely prescribed anti malarial in the tropics, and, though providing symptomatic relief, does cause nausea, blurred vision, hypotension and, in chloroquine poisoning, coma and dysrhythmias. Mefloquine resembles quinine in structure and remains

generally effective - it may, however, cause nausea and diarrhea. Halofantrine is effective against resistant *falciparum*, but has poor bioavailability and may not be used in pregnant women. Sulfadoxine-pyrimethamine is used only if chloroquine and quinine are not available, as there is widespread resistance to it. Quinghaosu, an artemisnin compound which are currently the most effective one due to their rapid action and few side effects, are use mainly in the contexts of chloroquine resistance malaria. However, even the newest drugs are not flawless; the side-effects are vomiting, pruritus, fever, bleeding and cardiac arrhythmias rarely occur. Despite reports of brainstem neurotoxicity with high doses in animal studies, this side effect has not been observed in humans to date. All patients stayed in the hospital till general condition improved. They were afebrile, gained appetite and reached parasite clear from the circulation. It took about 5 days from admission to discharge home. Patients were called for follow up on day 13 and day 20. Physical examination and a sample of blood were collected for hematocrit, reticulocyte counts and parasite counts, thick and thin films daily, platelet count was done on alternated days. Same schedule was repeated on day 13 and day 20.

3.6 Laboratory method

Measurement of Platelet count was done on a fully automated, quantitative Coulter AC.T Diff TM Analyzer. (Coulter Corporation, Beckman Coulter Company, Miami, Florida, USA). Platelet count was the number of thrombocytes derived from directly measured platelet pulses, multiplied by a calibration constant and expressed in thousands of thrombocytes per microliter of whole blood. Coefficient of variation (CV) for the platelet count was ≤7%. Baseline platelet counts were done on the day of presentation. Repeat platelet counts were done in subjects with marked thrombocytopenia until normal or near-normal values were reached.

3.7 Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) version 11 for window. 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-Simrnov) test. Data was expressed as means and SD. In case of data do not show normal distribution, the quantitative results will be expressed as geometric mean or median (min-max) (95%CI). However, if the distribution of data shows normality, the quantitative results will be expressed as arithmetic mean (SD). Qualitative data will be expressed as number of observations with percentages (%). Estimation and hypothesis testing were done by inferential statistics (i.e. univariate, bivariate, multivariate analysis). For the summarization of data descriptive statistics will be used.

3.8 Research Fund

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

CHAPTER V
RESULTS

A total of 220 patients fulfilled the inclusion criteria for this study. There were 110 patients were comprised to uncomplicated group and 110 patients were in severe group. Age fluctuated between 15 and 63 years with median value 25 for uncomplicated malaria group and that of severe group, it was 24 years old with a range of 15-70. There was a male predominance in both group , male and female ratio 1.5:1 for uncomplicated group where as 2.5: 1 for severe group. *Plasmodium falciparum* was diagnose in all of the patients from both group by blood film examination. When we observed severity with ethnic groups, there were Thai 47(21.4%), Hmong 35(15.9%), Burmese 6(2.7%) and Karen 22 (10%) in uncomplicated group and Thai 37(16.8%), Hmong 34(15.5%) , Burmese 15 (6.8%) and Karen 24(10.9%) in severe group and no statistical significant were found between ethnicity and severity. When we studied the resisdence province with different types of malaria 57(52%) from Tak province, 33(30%) from Kachanaburi province and 20(18%) from Ratchaburi province in uncomplicated group where in severe group 58(53%) from Tak, 42(38%) from Kanchanaburi and 10(9%) from Rachatburi province. From those data most of the patient is from Kanchanaburi province. Most of the patients (80%) were laborer and others were farmers and monks. When we observed between previous malaria infection and severity 61 (27.7%) of patients from uncomplicated group and 37(16.8%) of patients from severe group have a history of malaria infection before and statistical significant was found. The disease terms to be less severe in patients, who have history of malaria infection before. Hepatomegaly was observed in 15(6.8%) in uncomplicated group and 35(15.9%) in severe group and statically significant were found between severity and hepatomegly. Splenomegaly was observed in 9(4.1%) and 8(3.6%) in severe group but no stastical significant were found. Hyperparasitemia was found only in severe group that is 59(26.8%) and statically significant was found. (Table 1, Fig 1, Fig 2)

Table 1. Results uncomplicated and severe malaria patients in absolute and percentage value with their significant level.

	Uncomplicated malaria	**Severe malaria**	**P value**
Age	25(15-63)	24(15-70)	
Sex			
Male	64(29.1%)	80(36.4%)	
Female	46(20.9%)	30(13.6%)	
Ethnic group			
Thai	47(21.4%)	37(16.8%)	
Hmong	35(15.9%)	34(15.5%)	
Burmese	6(2.7%)	15(6.8%)	
Karen	22(10%)	24(10.9%)	
Residence province			
Tak	57(52%)	58(53%)	
Kachanaburi	33(30%)	42(38%)	
Ratchaburi	20(18%)	10(9%)	
Occupation			
Laborer	86(78%)	84(76%)	
Farmer	22(20%)	25(23%)	
Monk	2(2%)	1(1%)	
Previous malaria infection	61(27.7%)	37(16.8%)	0.001
Hepatomegaly	15(6.8%)	35(15.9%)	<0.001
Splenomegaly	9(4.1%)	8(3.6%)	0.801
Hyperparasitemia	0	59(26.8%)	<0.001

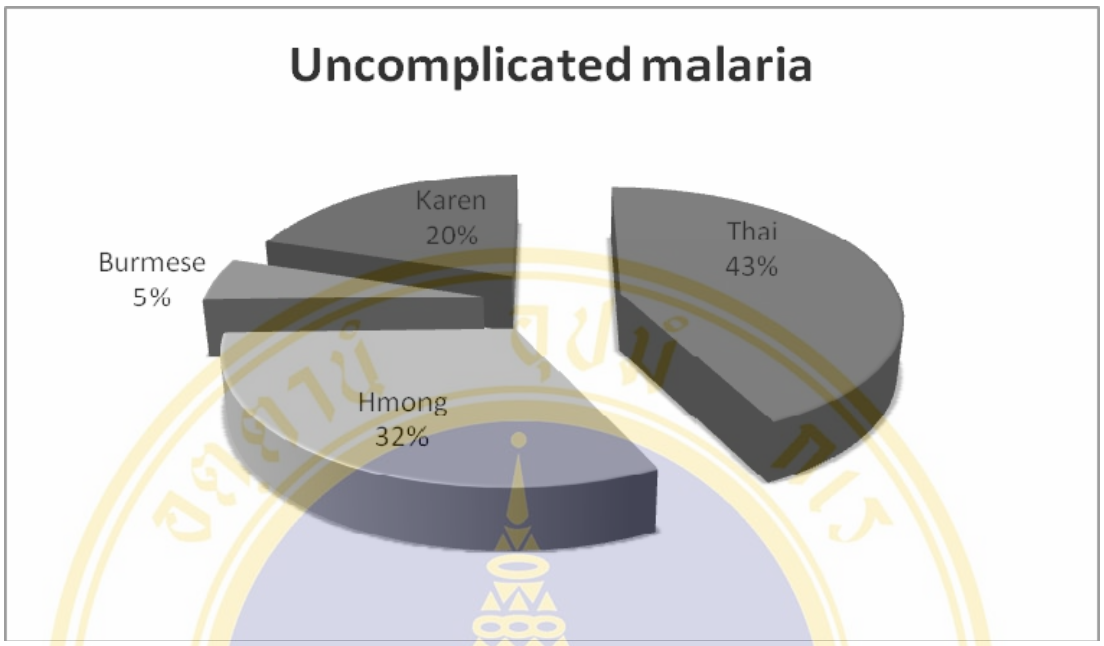

Figure 1. Distribution of different ethnicity by percentage in uncomplicated malaria patients

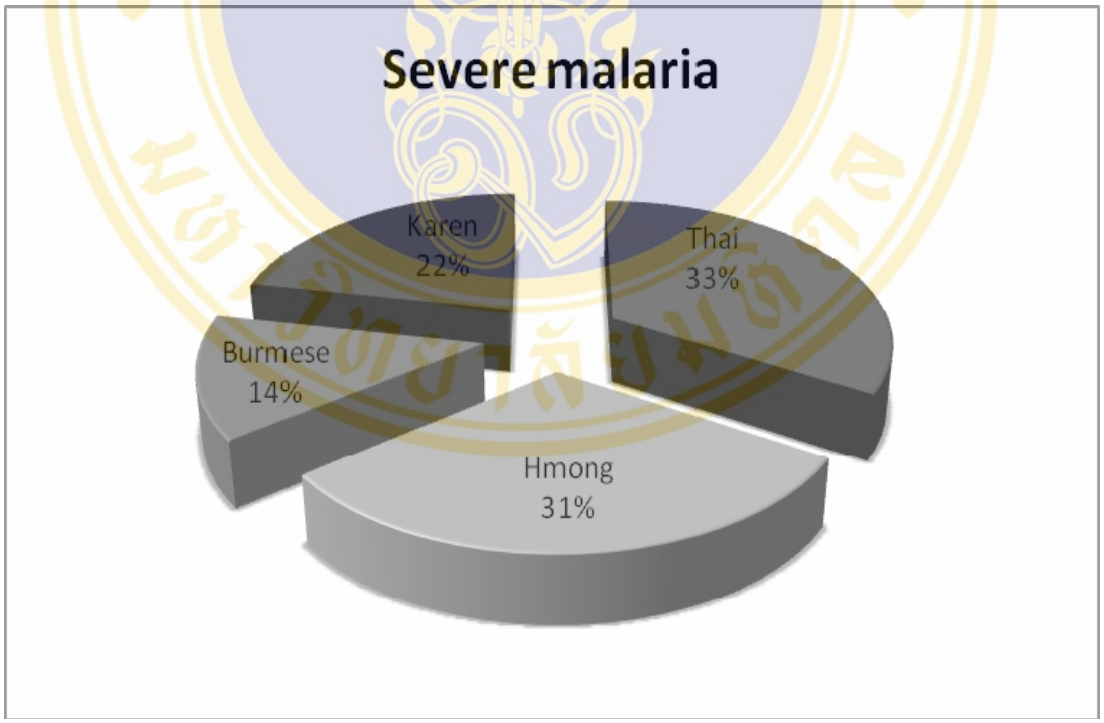

Figure 2. Distribution of different ethnicity by percentage in severe malaria patients

After that we try to find out is there any different between presentation sign and symptom of patients in both groups with severity and their significant level. (Table 2,Fig 3)

Table 2. Results of clinical findings in uncomplicated and severe malaria patients absolute and percentage value with their significant level

Clinical presentaion	Uncomplicated malaria	Severe Malaria	P value
Fever	110(50%)	110(50%)	
Chill and rigor	91(41.4%)	94(42.7%)	0.580
Myalgia	41(18.6%)	54(24.5%)	0.077
Arthralgia	2(0.9%)	3(1.4%)	0.653
Weakness	0	4(1.8%)	N/A
Anorexia	59(26.8%)	75(34.1%)	0.027
Vomiting	47(21.4%)	58(26.4%)	0.139
Nausea	46(20.9%)	61(27.7%)	0.043
Abdominal pain	20(9.1%)	20(9.1%)	1
Diahorrea	13(5.9%)	13(5.9%)	1
Jaundice	0	5(2.3)	N/A
Peripheral odema	0	1(0.5%)	N/A
Cough	9(4.1%)	12(5.5%)	0.491
Dyspnoea	0	0	N/A
Headache	96(43.6%)	92(41.8%)	0.444
Alteration of conscious	0	3(1.4%)	N/A
Convulsion	0	0	N/A

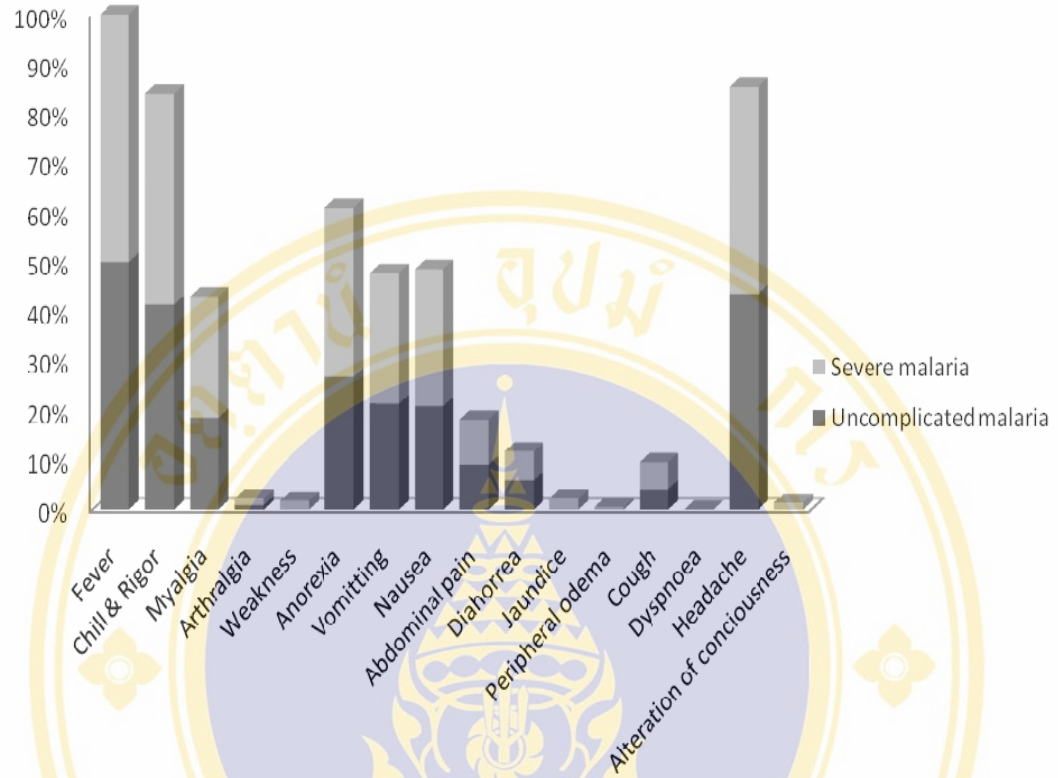

Figure 3. Presenting sign and symptoms at day of admission in both uncomplicated and severe malaria

When we observed baseline biochemical findings at the time of admission bicarbonate, sodium, blood glucose, direct bilirubin, AST, ALT, alkaline phosphatase and albumin show difference in uncomplicated and severe groups significantly ($p<0.001$ for each group). Nevertheless, potassium, choloride, total bilirubin, total protein and globulin were not significant. Regarding to AST and ALT severe patients had high level on baseline (>50 U/L and >60 U/L respectively) but they were still normal in uncomplicated patients (Table 3).

Table 3. Biochemistry data of patients in uncomplicated and severe malaria at admission day

	Uncomplicated malaria Mean (SD)	**Severe malaria Mean (SD)**	**P value**
HCO3	23.8(2.61)	20.7(2.59)	<0.001
Sodium	136(3.79)	133((4.18)	<0.001
Potassium	3.7(0.43)	3.61(0.69)	0.28
Chloride	101.2(9.22)	98.8(9.84)	0.62
Blood glucose	114.76(24.22)	132 (44.11)	<0.001
Total bilirubin	4.07(25.36)	6.8 (26.53)	0.435
Direct bilirubin	0.47(0.64)	1.7 (2.46)	<0.001
AST	43.8(40.48)	76.9 (74.35)	<0.001
ALT	38(35.04)	63.7(53.75)	<0.001
Alkaline phosphatase	90.3(41.42)	118.2 (63.24)	<0.001
Total protein	6.49(0.75)	8.6 (11.74)	0.164
Albumin	3.89(0.69)	3.4 (0.61)	<0.001
Globulin	2.97(0.50)	2.9 (0.47)	0.85

Table 4. Comparison of means value of Hematologic parameters for malarious subjects by sex

Parameter	Sex	No	Mean	P value	Preference value†
Hemoglobin (g/dl)	Male Female Different	144 76	12.6 10.9 2.3	<0.001	13.5-18.0 12.0-16.0‡
RBC	Male Female Different	144 76	4.6 4 0.6	<0.001	4.6-6.2 4.2-5.4
WBC	Male Female Different	144 76	5.6 6 -0.4	0.27	4.5-11.0 4.5-11.0
Platelet	Male Female Different	144 76	80 116 -36	0.001	150-400 150-400

† Mazza LL, 1995

‡ Anemia is sometimes defined as a hemoglobin level < 12 g/dL for men and < 10 g/dL for women. (Richards MW et al., 1998)

When we observed the hematological changes in different groups, on baseline, both group had decreased hemoglobin level lower than normal 11.5 mg/dl for uncomplicated and 11 mg/dl for severe group. One week after treatment, it reduced more than the baseline in both group of infections 11 mg/dl for uncomplicated and 10.7 for severe then it want up day by day and at the end, it become 12.4 mg/dl and 11.9 mg/dl respectively. In total leucocytes series, the baseline was just above the normal for both groups 5.48×10^9/L for uncomplicated and 6.11×10^9/L for severe and it become increased the level a few days and at the end of study it became 7.56×10^9/L for uncomplicated and 7.97×10^9/L for severe infection. The baseline platelet level were low for both groups 120×10^9/L for uncomplicated and 65×10^9/L for severe infections. Immediately after taking treatment, it became shoot up for 338×10^9/L for uncomplicated and 337×10^9/L for severe infection. 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 249×10^9/L and 258×10^9/L respectively. (Figure 8, Table 7)

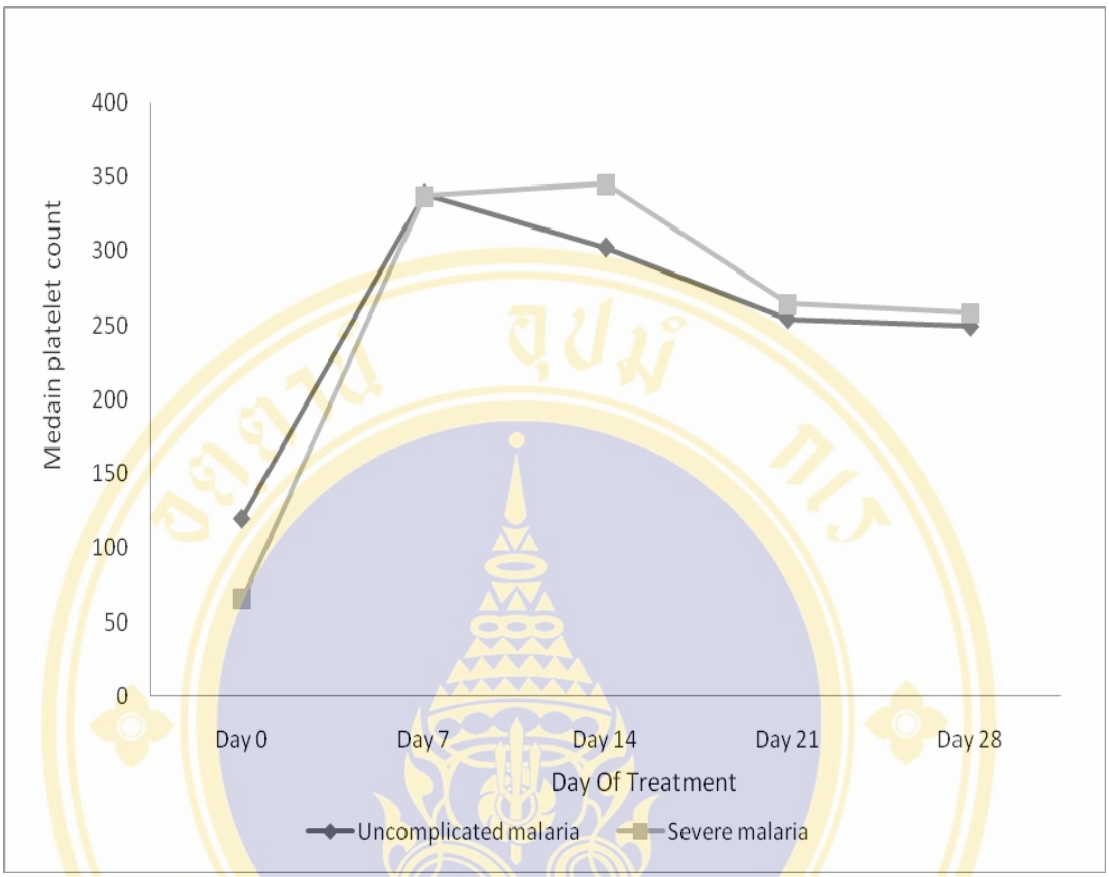

Figure 8. Median platelet count in relation to 10^9/L in uncomplicated and severe malaria patients from Day 0 to Day 28 (§,†= $p<0.05$)

Decrease level of platelet was observed on baseline but the platelet count became increase within first week after treatment. Then, it returned to normal slowly and stayed within normal at the end of the study in both groups. It still showed significant up to at the first week and it appeared again on the last day of the study period. (Figure. 8, Table.7)

Table 7. Hematological changes on Day 0, Day 7, Day 14, Day 21, Day 28 in uncomplicated and severe malaria patients with their significant level

	Uncomplicated malaria Mean (SD)	Severe malaria Mean (SD)	P value
Hb Day 0	11.5(2.41)	11 (2.08)	0.003
Hb Day 7	11(1.88)	10.7(1.38)	0.217
Hb Day 14	11.4(1.41)	10.6(1.81)	0.001
Hb Day 21	11.9(1.41)	11.3(1.76)	0.10
Hb Day 28	12.4(1.47)	11.9(1.72)	0.46
Hct Day 0	0.34(0.06)	0.37(0.06)	0.013
Hct Day 7	0.34(0.05)	0.33(0.52)	0.13
Hct Day 14	0.35(0.42)	0.33(0.53)	0.001
Hct Day 21	0.37(0.03)	0.35(0.45)	0.002
Hct Day 28	0.38(0.03)	0.45(0.54)	0.264
RBC Day 0	4.33(0.96)	4.58(0.75)	0.036
RBC Day 7	4.19(0.88)	4.07(0.63)	0.246
RBC Day 14	4.30(0.73)	3.9(0.70)	<0.001
RBC Day 21	4.44(0.70)	4.11(0.64)	<0.001
RBC Day 28	4.65(0.79)	4.31(0.54)	<0.001
WBC Day 0	5.48(1.80)	6.11(2.04)	0.05
WBC Day 7	7.61(2.17)	8.08(2.32)	0.127
WBC Day 14	7.77(2.25)	7.58(2.07)	0.51
WBC Day 21	7.53(2.20)	7.24(1.78)	0.28
WBC Day 28	7.56(1.66)	7.97(2.04)	0.11
Platelet Day 0	120(81.2)	65(73.11)	<0.001
Platelet Day 7	338(139.03)	337(129.10)	0.95
Platelet Day 14	302(108.83)	345(119.28)	0.006
Platelet Day 21	254(79.54)	264(77.23)	0.356
Platelet Day 28	249(84.65)	258(76.36)	0.459

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 of 11.5×10^9/L and 11×10^9/L was distributed between uncomplicated and severe group at the time of hospitalization that is Day 0. The changes of hemoglobin level affected equally to both groups and there was stastical significant (p=0.003). When we observed hemoglobin value for subsequent weeks, there was statistical significant at Day 14 (p=<0.001). The Level of hemoglobin in both groups was decreased in small amounts in Day 7 compare to Day 0. However, the hemoglobin level of both groups rise up to a certain level in the following days which started Day 21 in severe groups. In that case, both groups were compared and it was found that the difference were apparent.(Figure 4, Table 7)

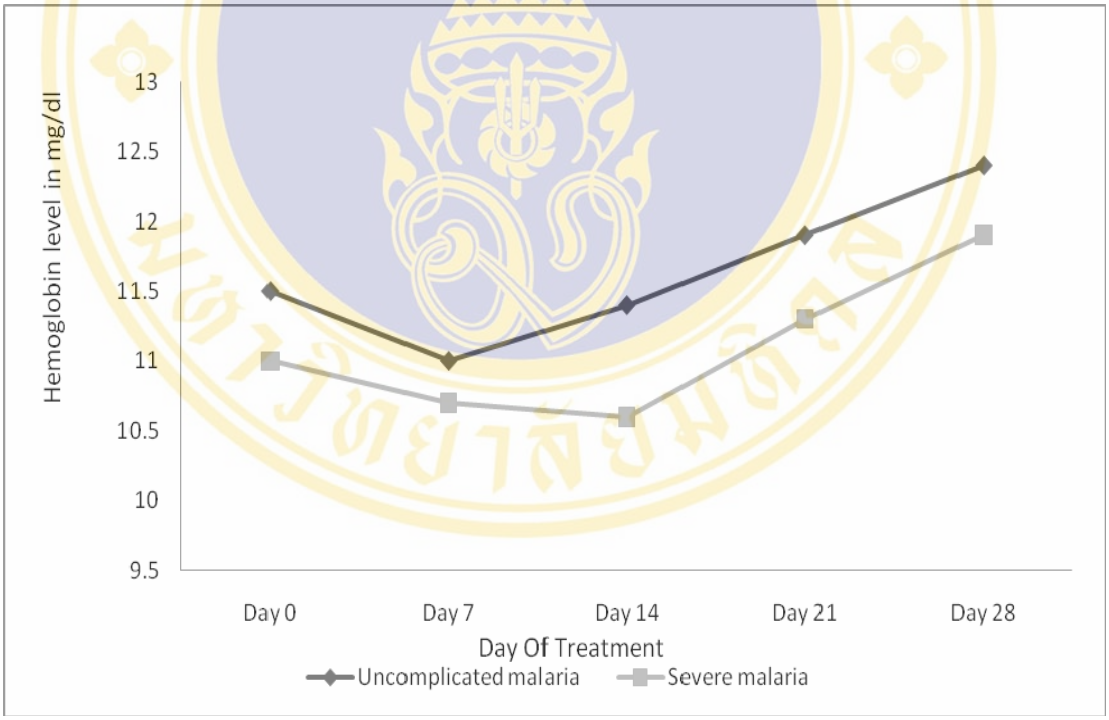

Figure 4. Median hemoglobin value in uncomplicated and severe malaria patients in mg/dl from Day 0 to Day 28 (§,† = p<0.05)

Secondly, hematocrit level of all patients was studied. The baseline result were 0.34 mg/dl for uncomplicated and 0.37 mg/dl for severe group there was significantly different in both groups (p=0.013). The median haematocrit level of both groups were analyzed up to Day 28. The degree of disease and respond to treatment was highly

depend on the level of haematocrit and it was significant for Day 0,14,21. 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 cases, it was found that the respond of uncomplicated patients were better and faster than that of severe group to touch normal. (Figure 5, Table 7)

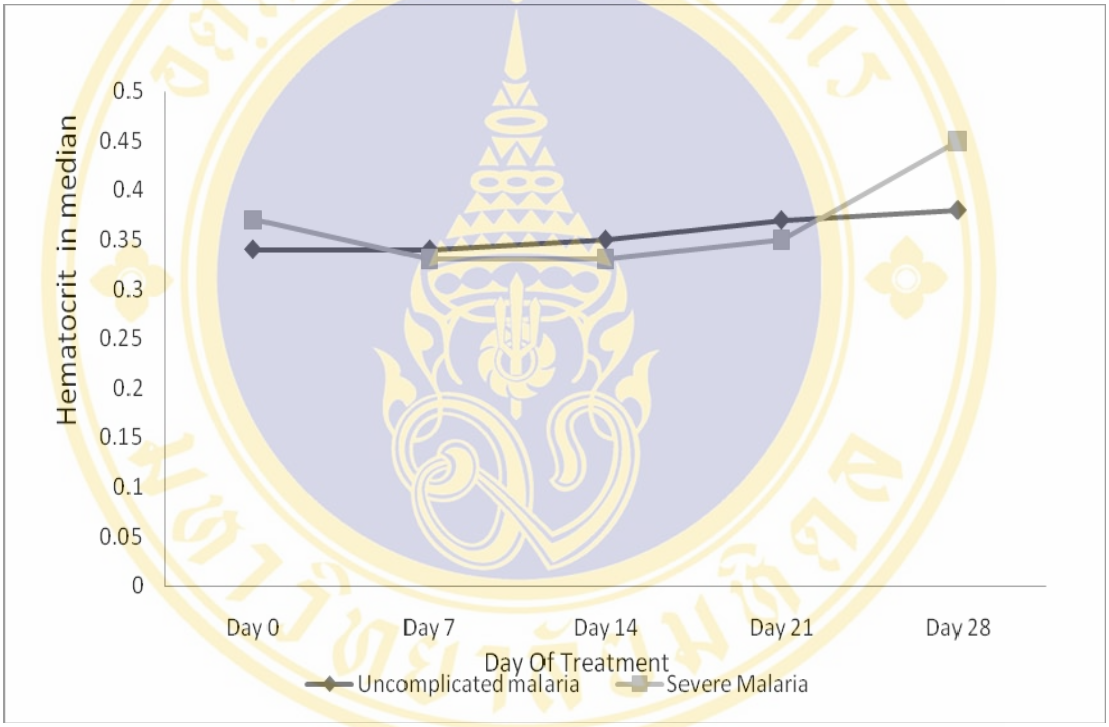

Figure 5. Median hematocrit value in uncomplicated and severe malaria patients from Day 0 to Day 28 (‡,†,§=p<0.05)

When RBC were analyzed, the median value of total RBC count on Day 0 4.33×10^9/L for patients with no complication and it was 4.58×10^9/L for patients with complication and there was no stastical significant. When it was studied continuously, it increased to a certain amount on Day 7 and Day 14. On Day 21 and Day 28 the level of both groups were nearly overlapped. All the changes in total WBC were within normal limit in the whole study. (Figure 6, Table 7)

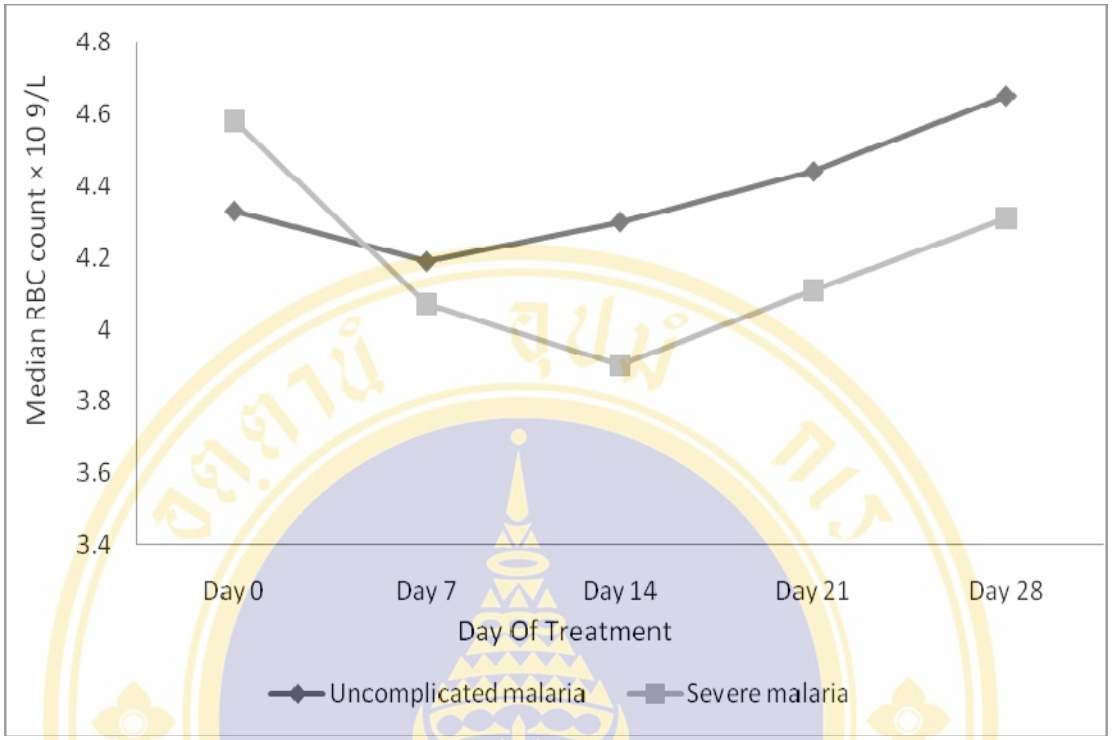

Figure 6. Median total RBC in relation to 10^9/L in uncomplicated and severe malaria patients from Day 0 to Day 28 (§,†,‡,¶ = $p<0.05$)

When white blood cells were analyzed, the median value of total WBC count on Day 0 5.48×10^9/L for patients with no complication and it was 6.11×10^9/L for patients with complication and there was no stastical significant. When it was studied continuously, it increased to a certain amount on Day 7 and Day 14. On Day 21 and Day 28 the level of both groups were nearly overlapped. All the changes in total WBC were within normal limit in the whole study. (Figure 7, Table 7)

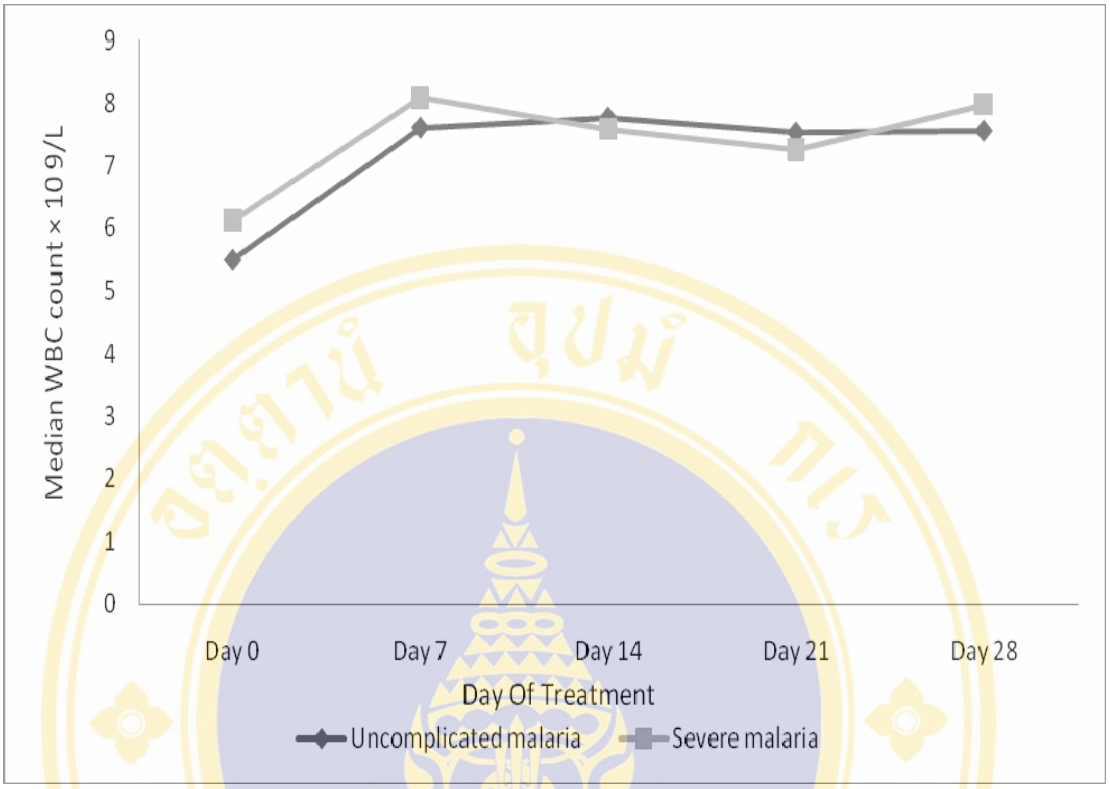

Figure 7. Median total WBC in relation to 10^9/L in uncomplicated and severe malaria patients from Day 0 to Day 28

Value of pearson correlation coefficient (p) and P.value are shown in Table 5 and 6 for both groups. Different variables' are analyses for possible correlation with platelet count at the time of admission (Day 0). For both groups we found statistical significant but weak negative correlation with platelet count Day 0 and severity pearson correlation coefficient = -0.338, p- value <0.001. There are also weak positive correlation between Gender (0.213), p-value 0.001, Hyperparsitemia (0.256), p-value <0.001 with platelet count at Day 0. When we observed biochemistry on admission with platelet count at day 0 we find weak positive correlation with HCO3 and sodium and albumin and weak negative correlation with direct bilirubin and AST.

Table 5. Relationship between platelet count at Day 0 with Age, Gender, Ethnicity, Past malaria infection, Hepatomegaly, Splenomegaly, Severity and Hyperparasitemia

	Platelet count at Day 0	
	Pearson correlation	P value
Age	0.002	0.973
Gender	0.213	0.001
Past malaria infection	-0.067	0.326
Hepatomegaly	0.104	0.124
Splenomegaly	-0.052	0.448
Severity	-0.338	<0.001
Hyperparasitemia	0.256	<0.001

Table 6. Relationship between platelet counts at Day 0 with Biochemistry at admission

Parameter	**Platelet count Day 0**	
	Pearson correlation	P value
HCO3	0.423	<0.001
Sodium	0.372	<0.001
Potassium	0.088	0.19
Chloride	0.153	0.24
Total bilirubin	-0.093	0.17
Direct bilirubin	-0.225	0.001
AST	-0.140	0.03
ALT	-0.117	0.08
Alkaline phosphatase	-0.120	0.07
Total protein	0.110	0.30
Albumin	0.193	0.004
Globulin	0.127	0.07

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, 220 patients were participated, 110(50%) uncomplicated and 110(50%) severe patients. When we observed the significant of the ethnic group, Hmong and Karen people were common and it was because all the patients who participated in the study come from the Burma-Thai border area especially those area are quite close to Hmong and Karen State. Hence, most people from these States come and worked in Kanchanaburi, Tak and Ratchaburi province. Naturally most of them were men and so there were male predominance in uncomplicated and severe groups. When the resisdence provinces were compared, there are no significant difference for the patients from Tak and Kanchanaburi and Rachaburi provinces regarding the severity of diseases. The reason of this could be whether these patients had some immunity to malaria or previously they get chemoprophylysis or the less number of patients from this province participated compared to other province 57(52%) from uncomplicated,58(53%) from severe group were from Tak and 33(30%) from uncomplicated ,42(38%) from severe group were from Kanchanaburi. The result of previous history of malaria showed that 61(27.7%) of uncomplicated patients had experience about the disease which was more than that of for other group 37(16.8%) 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 individual 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 chemoprophylysis 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. *Falciparum* infections are common in south east asia. Moreover, we know that these infections are common for

hepatosplenomegaly. In this research 15(6.8%) from uncomplicated and 35(15.9%) from severe had enlarged liver and 9(4.1%) from uncomplicated and 8(3.6%) from severe group had spleen respectively. Some of them had both liver and spleen enlargement.

Additionally, the baseline hemoglobin level of female patients was lower than that of 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.

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 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 biliubin might be due to hemolysis. The baseline albumin level for both complicated and uncomplicated group were significant be low albumin level was more pronounced in female patients. The significant 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.

When hematological data were studied for the whole study from day of admission to day of discharged the median hemoglobin level of uncomplicated patients showed less than 12 mg/dl in first week but it started increased gradually in the subsequent week 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. 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 hamatocrit level in both groups as hemoglobin level did.

The median values for total WBC count were analyzed for the whole study, it showed that low level of WBC count but more than 5×10^{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. All changes of total WBC in the whole study period occurred within the normal level($5 – 10\times10^9$/L).

Low platelet count found consistently for both uncomplicated and severe patients as baseline but for severe patients, the level was very low and which shown significant. However, within one week after getting treatment, the level shot up to nearly (350×10^9 / L) for both groups. For uncomplicated patients, it went down linearly up to upper normal (250×10^9 / L) in Day 28 but for sever patients, thrombocytosis still present Day 14 and then it gradually down up to normal in Day 28. The finding of low platelet count on first day agreed with other study. The mechanism could be peripheral destruction by macrophages or much platelet adhesion to both parasitized 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 is no DIC patients is not present in this study.

Thrombocytopenia, which occurred in more than half of the patients, was a characteristic finding. Other studies malaria has also reported that at least 50% of the malaria patients had thrombocytopenia, while only 28% of them had anemia. It appears from these different studies that thrombocytopenia may be more common than anemia in acute malaria infection. In the study of severe malaria cases in the Gizan area, thrombocytopenia was a common complication (50.4%). Thrombocytopenia is a classical feature of malaria, and a low platelet count is usually seen in about 85% of patients with uncomplicated malaria and all patients with severe *falciparum* malaria. It is so characteristic of malaria that in some places, it is used as an indicator of malaria in patients presenting with PUO. It has also been observed that there is an inverse relationship between platelet count and parasite level. It has also been reported that thrombocytopenia occurs early in illness and resolves within a few days of treatment. The precise mechanism behind thrombocytopenia, however, remains unclear. Decreased thrombopoiesis can be excluded because platelet-forming megakaryocytes in the marrow are usually normal or increased. Immune-mediated destruction of circulating platelets has been postulated, and it has been found that

malaria patients have elevated levels of platelet- bound IgG. Some workers have suggested DIC as a major mechanism, but others have found no evidence of DIC in any of their patients, including those with severe thrombocytopenia. Another proposed mechanism is that of platelets engulfing malaria parasites, and in the process becoming damaged and thus being removed from circulation. This has not been confirmed. Hypersensitive platelets have been found in acute malaria infection as well as additional changes in platelet function. These include raised concentrations of platelet specific proteins such as beta thromboglobulin (bTG), platelet factor 4 (PF4), and other changes such as enhanced production of thromboxane A2 and prostacyclin, but spontaneous platelet aggregation did not occur in these studies. It has also been postulated that these hypersensitive (hyperactive) platelets will enhance hemostatic responses, and maybe this is why bleeding episodes are rare in acute malaria infection, despite the thrombocytopenia. There are conflicting reports as to the frequency of abnormalities of the coagulation mechanism in malaria. Prolonged PT, depletion of blood coagulation factors V, VII, VIII, IX and DIC have been reported. Our patient recovered with treatment of malaria, however, and no further intervention was needed. In conclusion, the hematological aspects of malaria infection constitute a very interesting area in various reports. Mainly anemia and thrombocytopenia are the classical changes. Changes in the white blood cell are less dramatic and there has been conflicting reports regarding these changes. It would be interesting to study further and compare the different reports discussing the hematological findings in both immune and semi-immune patients living in endemic areas, and those returning with imported malaria. It would also be beneficial to carry out prospective studies on other aspects, which have not been conclusive, e.g., coagulation parameters, bone marrow changes and the direct antiglobulin test (to help in understanding the anemia). In fact, results of direct antiglobulin tests and ^{51}Cr red cell survival studies have shown some evidence for increased destruction of non-parasitized and parasitized erythrocytes by possible immune-mediated mechanisms. Laboratory and clinical studies, however, failed to establish the presence of antibodies on erythrocyte surface and hemolysis in malaria infected patients.

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* infection contributed to most of the changes such as low hemoglobin level and platelet level in baseline. We do a lot of study of correlation between uncomplicated and severe malaria in hematology. Some of our findings were consistent with previous studies although some were against to others. Principally, anemia on baseline, persistent lowering of baseline thrombocytopenia followed by thrombocytosis are the main changes in this study. Platelets count was very low at the day of admission and it want up within one week of treatment and up until normal at the end of the study. No intervention such as blood transfusion or platelet transfusion was needed to make the platelet return to normal level. 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 cause that can affect to hematological parameters.

As conclusion, these findings in this study are sincerely expected to be useful to many clinicians and heath care personal 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

Study No ☐☐☐☐
Date of Admission (mm/dd/yyyy)_____/_____/_____(hh/mm)____:____

Demographic data
Age: (Yrs) ____(mos)_____
Date of birth (mm/dd/yyyy) _____/_____/_____

Gender (1) Male ☐
(2) Female ☐
(9) No Data ☐

Ethnicity (1) Thai ☐ (3) Burmese ☐ (5) Other _____
(2) Hmong ☐ (4) Karen ☐ (9) No data ☐

Residency (1) Bangkok district ☐ (3) Other district ☐
(2) Mae Sot district ☐ (4) Other Province ☐ (9) No data ☐

Occupation (1) Farmer ☐ (3) Office/Government ☐ (5) Other _____
(2) Labour ☐ (4) None ☐ (9) No data ☐

Past medical history
Previous malaria infection (1) Yes ☐ (2) No ☐ (9) No data ☐

History of chronic illness (1) Yes ☐ (2) No ☐ (9) No data ☐

If Yes
(1) Hypertension ☐ _____
(2) Diabetes mellitus ☐ _____
(3) Kidney disease ☐ _____
(4) Liver disease ☐ _____
(5) Pulmonary disease ☐ _____
(6) Heart disease ☐ _____
(7) Neurological disease ☐ _____
(8) HIV positive ☐ _____
(9) Other ☐ _____

Long term medication (1) Yes ☐ (2) No ☐ (9) No data ☐
Drugs _____

Patient ID No _____
Physical examination (mm/dd/yyyy) _____/_____/_____

(1) Jaundice	(1) Yes ☐	(2) No ☐	(9) No data ☐
(2) Anemia	(1) Yes ☐	(2) No ☐	(9) No data ☐
(3) Splenomegaly	(1) Yes ☐	(2) No ☐	(9) No data ☐
(4) Hepatomegaly	(1) Yes ☐	(2) No ☐	(9) No data ☐
(5) Peripheral oedema	(1) Yes ☐	(2) No ☐	(9) No data ☐
(6)Impaired consciousness	(1) Yes ☐	(2) No ☐	(9) No data ☐
(7)Convulsions	(1) Yes ☐	(2) No ☐	(9) No data ☐
Coma	(1) Yes ☐	(2) No ☐	(9) No data ☐

Other _____

Spontaneous Bleeding during admission (1) Yes ☐ (2) No ☐ (9) No data ☐
If Yes When _____/_____/_____

Any transfusion (1) Yes ☐ (2) No ☐ (9) No data ☐
If Yes When _____/_____/_____
Type _____
Indication _____

Chest X- Ray (1) Yes (2) No (9) No data

If Yes (mm/dd/yyyy) ____/____/____

Finding
(a) Pulmonary oedema (1) Yes ☐ (2) No ☐
(b) Infiltrations (1) Yes ☐
If yes specify: _____
(2) No ☐
(c) Other (1) Yes ☐
If yes specify: _____
(2) No ☐

Criteria for severe malaria (WHO 2006)

(1) Impaired consciousness	(1) Yes ☐	(2) No ☐	(9) No data ☐
(2) Severe pallor/ anemia	(1) Yes ☐	(2) No ☐	(9) No data ☐
(3) jaundice/ Hyperbilirubemia	(1) Yes ☐	(2) No ☐	(9) No data ☐
(4) Circulatory collapse	(1) Yes ☐	(2) No ☐	(9) No data ☐
(5) Metabolic acidosis	(1) Yes ☐	(2) No ☐	(9) No data ☐
(6) Pulmonary odema/ ARDS	(1) Yes ☐	(2) No ☐	(9) No data ☐
(7)Multiple convulsion	(1) Yes ☐	(2) No ☐	(9) No data ☐
(8) Spontaneous bleeding	(1) Yes ☐	(2) No ☐	(9) No data ☐
(9) Hemoglobinuria	(1) Yes ☐	(2) No ☐	(9) No data ☐
(10)Hypoglycemia	(1) Yes ☐	(2) No ☐	(9) No data ☐
(11) Hyperparasitemia	(1) Yes ☐	(2) No ☐	(9) No data ☐
(12) Prostration	(1) Yes ☐	(2) No ☐	(9) No data ☐

Patients ID No _____

Presenting Symptoms (1) Yes ☐ (2) No ☐ (3) No data ☐

If Yes :

	Symptoms / Sign		**Duration of illness**
1	Fever		
2	Chill/ Rigor		
3	Myalgia		
4	Arthralgia		
5	Fatigue		
6	Anorexia		
7	Vomiting		
8	Abdominal discomfort		
9	Diarrhoea		
10	Jaundice		
11	Peripheral oedema		
12	Cough/ Dyspnoea		
13	Headache		
14	Alteration of consciousness		
15	Convulsions		
16	Coma		
17	Other		
18	Other		

Biochemistry	**Unit**	**Day** ____/____/____
Serum pH	-	
HCO_3	mmol/l	
Sodium	mmol/l	
Potassium	mmol/l	
Chloride	mmol/l	
Calcium	mg%	
Blood glucose	mg%	
Bilirubin total	mg%	
Direct Bilirubin	mg%	
AST	U/L	
ALT	U/L	
Alk. Phosphatase	U/L	
Total protein	g%	
Albumin	g%	
Globulin	g%	
Myoglobin		
CPK	U/L	
LDH	U/L	
Urine Analysis		
Urine color	-	
Urine pH	-	
Urine Albumin	+/_	
Urine protein	mg/dl	
Urine specific gravity	g/l	
UPCI		
Urine RBC	/HPF	
Urine WBC	/HPF	

Patient ID	Date	Day 0	Day 7	Day 14	Day 28	Any Bleeding
		___/___/___	___/___/___	___/___/___	___/___/___	___/___/___
Temperature	°C					
Pulse rate	/min					
Blood pressure	mmHg					
Intake oral	ml/24hr					
Intake total	ml/24hr					
Output urine	ml/24hr					
Output ultrafiltration	ml/24hr					
Output total	ml/24hr					
Haematology						
Hb	g%					
Hct	%					
RBC	$\times 10^2$/L					
WBC	$\times 10^6$/L					
Neutrophil	%					
Lymphocyte	%					
Platelet count	$\times 10^9$/L					
P.f/ P.v/ mix infection	-					
G6PD						
Hb-Typing						
PT	sec					
PTT	sec					
INR						

Study No _____

Outcome

(1) Survival Date of hospital discharge (mm/dd/yyyy) ____/____/____

(2) Death Date of death (mm/dd/yyyy) ____/____/____ (hr/min)___/___

If death suspected causes of death

1	Cerebral malaria	
2	Renal failure	
3	Acidosis	
4	Hyperkalaemia	
5	Respiratory failure	
6	Cardiac/ Circulatory failure	
7	Liver failure	
8	2^{nd} ary infection/ sepsis	
9	Abnormal bleeding / DIC	
10	Multi-organ failure	
11	Other	
99	No data	

BIOGRAPHY

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