EMERGENCE AND CLEARANCE OF GAMETOCYTES IN COMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Thematic paper
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ABSTRACT

The purpose of this retrospective study was to determine the prevalence of gametocytemia in complicated *Plasmodium falciparum* malaria. Data were collected by reviewing 531 hospital charts of patients admitted to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, in Bangkok, in the period January 2000-December 2006. All admitted patients received standard antimalarial medication. Blood films were checked daily until discharge.

Circulating gametocytes were observed in 254 (47.8%) of patients and in most cases (221/254; 87%) gametocytemia was detected during the first 24 hours post-admission.

Gametocytes were first seen in 164 (64.6%) patients on admission, 42 (16.5%) at 12 hours, and 15 (5.9%) at 24 hours post-admission.

The longest interval between admission and first appearance of gametocytes was 348 hours. The median gametocyte clearance time was 105 hours (range = 2-655) for patients whose gametocytemia resolved. However, 4 patients (1.6%) were discharged with gametocytemia.

In general, gametocytemia presented within the first 24 hours post-admission and, emerged in only 13% of patients later, during artemisinin treatment.

There was no association between parasite clearance time and gametocyte clearance time.

KEY WORDS: COMPLICATED *PLASMODIUM FALCIPARUM* MALARIA/ GAMETOCYTES/ EMERGENCE/ CLEARANCE

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LIST OF ABBREVIATIONS

Abbreviation of Symbol	Term
%	Percent
ACT	Artemisinin-based Combination Therapy
ARDS	Acute Respiratory Distress Syndrome
BMI	Body mass index
°C	Degree Celsius
DIC	Disseminated Intravascular Coagulation
dl	Deciliter
FCT	Fever Clearance Time
GCT	Gametocytes Clearance Time
G	Gram
GI	Gastrointestinal
G6PD	Glucose-6-Phosphate Dehydrogenase
ICAM1	Intercellular adhesion molecule
i.e.	Id est
IL	Interleukin
mg	Milligram
µL	Microliter
mL	Milliliter
mmol	Millimol
PCT	Parasite Clearance Time
P.	Plasmodium

LIST OF ABBREVIATIONS (CONT.)

Abbreviation of Symbol	Term
PfEMP1	Plasmodium falciparum erythrocyte membrane protein 1
RBC	Red Blood Cell
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
TNF	Tumor Necrosis Factor
vs.	versus
WBC	White Blood Cell
WHO	World Health Organization

CHAPTER I
INTRODUCITON

Malaria is caused by obligate intraerythrocytic protozoa of the genus *Plasmodium.* Human can be infected with one or more of the following four species *P. falciparum, P. vivax, P. ovale* and *P. malaria*.

Malaria has known to be one of the world's major causes of death. The manifestation of malaria has shown different pattern this deference come from many factors, such as plasmodium species, geographical distribution, drug treatment and immunity. *P. falciparum* is the most common cause of severe and life threatening malaria, resistant to many currently available antimalarial drugs. Falciparum malaria causes over one million deaths every year (WHO, 2006a). In Africa, a vast majority of these deaths occur in children under five years of age and pregnant women (White, 2007). The presentation of severe malaria varies with age and geographical distribution.

During 1980s and 1990s the number of cases of malaria in Africa increases because of the increasing risk of transmission in areas where malaria control has declined (WHO, 2005), as well as in India between 2003 and 2005 (Singh et al., 2006). During the past decade, malaria also resurged or increased in intensity in South-East Asia after interruption of eradication efforts, and re-emerged in several Central Asian and Transcaucasia countries (WHO, 2005).

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 to 30000 travelers from industrialized countries are expected to contract malaria each year (Lo, III and Gluckman, 2003; Franco-Paredes and Santos-Preciado, 2006). In addition, drug resistant *P. falciparum* malaria continues to spread and at present involves almost all areas in the world. The rapid emergence and spread of drug resistance are major problems for malaria control.

An increasing number of travelers are exposed to drug resistant plasmodia. Reducing of transmission is therefore important both to control malaria and to delay the spread of drug resistance.

Transmission of malaria from infected humans to the mosquito involves the production of gametocytes which are the sexual stage of the malaria parasite. They are produced in the human host (gametocytogenesis) but remain in a state of arrested cell development until being ingested by *Anopheline* mosquito. Male micro-gametocytes are released in the insect's midgut and fertilize activated female macro-gametes (Talman et al., 2004). The subsequent development of mosquito specific stage results in infection of the mosquito salivary glands and with sporozoite and render the mosquito infectious to humans. Gametocytes are thus vital to the completion of the malaria transmission cycle.

Although *Plasmodium* parasites were discovered more than century ago, the exact mechanism of gametocytogenesis is not well defined. Previous studies have been shown several factors influencing gametocyte emergence. Without effective treatment, most patients with *P.falciparum* malaria can develop gametocytemia within 10-40 days after the onset of parasitemia (Collins and Jeffery, 1999).

Although antimalarial drugs can reduce transmission in falciparum malaria indirectly by reducing the number of asexual parasites that could subsequently develop into sexual stages, most of antimalarial drugs have no direct effects on mature gametocyte. The only drug has been proven to have gametocidal activity is primaquine.

Prevalence of gametocytemia and gametocytes clearance in uncomplicated *P. falciparum* malaria has been defined by several studies. However, little is known about prevalence of gametocytemia in severe falciparum malaria infection. Therefore, this study aims to determine the prevalence of gametocytemia, the emergence and clearance of gametocyte in severe falciparum malaria infection.

CHAPTER II
OBJECTIVES

Primary objectives

This retrospective study was to determine the prevalence of gametocytemia in severe *P. falciparum* malaria patients admitted to Bangkok Hospital for Tropical Diseases between years 2000 to 2006.

Secondary objectives

The secondary objectives of this study were to

- Determine gametocyte emergence and clearance time
- Find association between parasite clearance time and gametocyte clearance time in patient with complicated malaria.

CHAPTER III
LITERATURE REVIEW

Malaria is protozoan infection of human red blood cells transmitted by bite of female *Anopheline* mosquito, it regards as major health problem in tropical countries and the clinical case load may exceed 500 million. It leads to about 1 to 3 million deaths mainly among young children and more than 90% of this death occurs in sub-Saharan Africa (Pasvol, 2006).

Geographical distribution has been known throughout tropics where are environment is suitable for occurrence of infection, *P.falciparum* which is predominant in Africa and Papua New Guinea and Haiti, while *P. vivax* most common in central part of south America, north Africa, middle east, India and it is very rare in sub Saharan Africa. And both of them are nearly equally distributed in Asia and other part of South America. *P. ovale* is almost all confined to West Africa (White, 2007).

Pathophysiology of malaria results from integration of many processes. Certain factors has been contributed such as cytokines (IL1, IL4, TNF …), host immunity, parasite factors such as (PfEMP1). These factors lead to sequestration of some of the infected RBC, rosettes formation, deformability, and aggregation of RBC to micro vascular endothelium in many organs such as brain, lung spleen, liver, and kidney. This feature unique to *Plasmodium falciparum*

Rosetting is adherence of infected RBC with uninfected RBC; aggregation is adherence of infected RBC together mediated by platelet. Deformability defined as loss of RBC flexibility and change of RBC membrane from fluidity to rigidity and shape from biconcave to spherical (Dondorp et al., 2004). All these factors lead to

impaired microcirculatory flow which acts as bases of pathophysiology of severe malaria (Dondorp et al., 2000).

Malaria has been classified into uncomplicated and complicated malaria according to world health organization criteria(WHO, 2006b). The most complications are due to *P.falciparum* infections which include:

- Cerebral malaria (unrousable coma): unrousable coma not attributable to any other cause in a patient with falciparum malaria. Coma should persist at least 30 minutes after a generalized convulsion to make the distinction from transient post-ictal coma.
- Severe normocytic anemia: normocytic anemia with hematocrit less than 15% or hemoglobin less than 5 g/dL in the presence of parasitemia more than 10,000 parasites per μL. If microcytic indices seen, need to consider iron deficiency anemia, thalassemia and hemoglobinopathy.
- Renal failure: urine output less than 400 mL in 24 hours in adults, or 12 mL per kg in children, failing to improve after rehydration, and with serum creatinine more than 265 μmol/L (3 mg/dL).
- Pulmonary edema, ARDS: Tachypnoea, dyspnoea and bilateral basal rales.
- Hypoglycemia: whole blood glucose less than 2.2 mmol/L (less than 40 mg/dL).
- Circulatory collapse, shock: hypotension (systolic blood pressure less than 50 mm Hg in children from one to five years old; less than 70 mm Hg in adults) with cold, clammy skin or a core to skin temperature difference more than 10 °C.
- Spontaneous bleeding, DIC: spontaneous bleeding from gums, nose, GI tract or other sites, with laboratory evidence of DIC.
- Repeated generalized seizures: more than 2 observed seizures within 24 hours despite cooling.
- Acidemia or acidosis: arterial pH less than 7.25, plasma bicarbonate less than 15 mmol/L.

- Malarial hemoglobinuria: need to exclude hemoglobinuria due to antimalarial medications and to G6PD deficiency.
- Impaired consciousness but rousable: impaired consciousness less marked than unrousable coma, can localize a painful stimulus.
- Prostration and extreme weakness: patient unable to sit or walk, with no other obvious neurological explanation.
- Hyperparasitemia: very high parasite densities are associated with increased risk of severe disease but is affected by the immune status (more than 5% parasitemia in non-immune is serious, but may be well tolerated in semi-immune children); more than 500,000 per μL.
- Jaundice: total bilirubin more than 50 μmol/L (more than 3 mg/dL).
- Hyperpyrexia: rectal temperature more than 40°C.
- Post-mortem evidence of severe malaria: neuropathologic evidence of venules and capillaries packed with erythrocytes containing malarial parasites.

All malaria parasites require the presence of two hosts to complete their life cycle, definitive host (in which sexual development occur) is the *Anopheline* mosquito and the mammal is the intermediate host. The parasites adopt three very different cellular strategies in the distinct phases of the complex life cycle (David A.Warrell and Herbert M.Gilles, 2002). The first strategy is ability to grow replicate extensively (schizogony) this is achieved by three stages: oocyst in the mosquito (where the process is called sporogony); tissues schizogony (also termed exo-erythrocyctic schizogony or pre-erythrocyctic schizogony) in the liver of mammalian host; and erythrocyctic schizogony. The second strategy is invasion of the host cells. The parasite stages adopting this strategy are the extracellular and are the merozoite, sporozoite and the ookinete. The third strategy is the sex, which begins with the formation of gametocyte in the peripheral circulation of the vertebrate host and is completed upon formation of ookinete in the mosquito blood meal.

Gametocytes mainly arise from erythrocyctic asexual stages (Figure1). It's well established that the ratio of gametocytes to asexual stage in *P. falciparum* is very low, recent study calculates a ratio of (1:156) (Eichner et al., 2001). The production of

gametocytes directly from hepatic merozoite, which has been described in other species, may occur in *P. falciparum* (W.H.Wernsdorfer Sir I.McGregor, 1988). There has been much debate on the actual point of sexual differentiation and Bruce and colleagues (Bruce et al., 1990) have shown that merozoite emerging from a single schizont developed either into further asexual stages or into gametocytes. It has been further shown that the gametocytes from one schizont are all male or all female (i.e. not mixture) (Smith et al., 2000; Silvestrini et al., 2000; David A.Warrell and Herbert M.Gilles, 2002). This suggests that the trophozoite of the preceding asexual generation were already committed to either sexual development or continuing asexual cycling.

Malaria transmission depends mainly on the production of viable sexual stages of the parasite (gametocytogony) and their appearance in sufficient numbers in the circulation of human host (Price et al., 1999), it differs according to parasite species e.g. 4 days in *P. vivax* and 7-10 days in *P. falciparum* (White, 2007). So gametocytes acts as corner stone in malaria transmission to the mosquito vector (Drakeley et al., 2006). Gametocytogenesis in falciparum malaria is delayed until asexual stage has developed and is dependent on both asexual parasite densities and the duration of infection, it is thought not to start at same time with asexual cycle, but it takes time to develop. So the longer the infections with *P. falciparum* malaria the more likely to produce gametocytes

The exact mechanism of gametocytogenesis is unknown (Dyer and Day, 2000), but several factors influencing gametocyte emergence have been proposed including parasite genetics (Graves et al., 1984), stress to parasite (Alano and Carter, 1990), host hormonal factors (Lingnau et al., 1993), host immunologic factors (Drakeley et al., 1999), seasonal variation (Nacher et al., 2004), patient age (Bousema et al., 2004). The mechanisms that have been explained why sexual density of the parasite is low relatively to those of asexual parasitemia are:

- Observed densities are determined by host immune responses, with high numbers of gametocytes being rapidly cleared and gametocyte densities are

determined by the rate at which they are produced (reproductive restraint) (Talman et al., 2004).

- Another mechanism have been proposed to explain low gametocytemia across stage immunity to PfEMP-1 by immunoglobulin G antibodies which effect on malaria transmission by regulating production of gametocyte this regulation achieved either by controlling asexual proliferation and density or by affecting gametocyte maturation (Piper et al., 1999).

In laboratory study, temperature was associated with greater effects on controlling gametogenesis in *P. falciparum*, exflagellation take place most rapidly between 28 and 36°C, below this temperature there is an increasing delay of exflagellation, and below about 10°C exflagellation is totally arrested. Emergence and exflagellation of gametocytes of *P. falciparum* are totally arrested at 38 °C and above (W.H.Wernsdorfer Sir I.McGregor, 1988).

Certain factors have been proposed to affect the appearance of gametocytemia (Figure 1). In study done by Price *et al* in 1999 in uncomplicated *P. falciparum* malaria patients receiving various antimalarial drugs (either halofantrine or mefloquine or Artemisinin derivative or quinine), they could identify pure *P. falciparum* infections, anemia on presentation, recrudescent infections and prolonged preadmission history of fever could affects the appearance of gametocytes. The last three factors regard as indicators of a prolonged infection. Presence of gametocyte on admission can lead to prolong gametocytes carriage (Suputtamongkol et al., 2003). Patient's Age was found to be associated with gametocyte carriage in study conducted in Kenya. In this study age below five old has been shown to be risk factor for harbour gametocytemia (Bousema et al., 2004) .

The morphological appearance of *P. falciparum* gametocyte (Figure 2) has been described by field and shute as five different stages of maturation, stage I to stage V by light and electron microscope (Talman et al., 2004). Gametocytes stages I to IV (Figure 2 No.7-17) have been observed sequestered preferentially in the bone marrow and spleen same as asexual stage (MacPherson et al., 1985), whilst stage V

are released in the peripheral circulation and only become infectious to mosquitoes after a further two or three days of circulation (Smalley and Sinden, 1977), which is the stage of our concern.

The infectivity of the malaria parasite to the mosquito vector is determined by the availability of the gametocytes, the vector and their intrinsic capacity to infect. Generally there were two pattern of infectivity of the gametocyte to the vector may be recognized among infection with different plasmodium species first those that become infectious before the first peak of asexual parasitemia like *P.vivax* in this category gametocyte rapidly reach maturity, and second those that do not become so until after the first asexual remission and this group of species characterized by delay in infectivity beyond the first appearance of asexual parasite in the blood and *P.falciparum* situated in this group (W.H.Wernsdorfer Sir I.McGregor, 1988).

Different classes of antimalarial drugs exert characteristic effects upon the infectivity of gametocytes to mosquitoes. They may divide into three major groups (W.H.Wernsdorfer Sir I.McGregor, 1988):

- Those which are lethal to the mature gametocytes within the host blood circulation.
- Those which exert their effects against the sporogonic stages following ingestion by the mosquito but do not affect the viability of the gametocytes within the host.
- Those which prevent the development of immature gametocytes but are without effect against the mature sexual forms either within the host or in the mosquito.

According to the classification above mature gametocytes (Figure 2 No.18-23) are relatively resistant to most antimalarials compounds like chloroquine, artemisinin derivative, and quinine. Primaquine is an only antimalarial drug that acts on mature infective gametocytes in the circulation and accelerates gametocyte clearance (Pukrittayakamee et al., 2004; WHO, 2006a). Artesunate has been shown to prevent

the maturation of immature *P. falciparum* gametocytes, this effect may contribute to reducing malaria transmission (Chotivanich et al., 2006).

The prevalence of gametocytemia in uncomplicated *P.falciparum* malaria has been reported in many studies. Piyaphanee *et al.* in 2006 could have found prevalence of 20.2%, majority of the gametocyte in their study was appear in the first 24 hours after admission and the longest period between admission and appearance is eight days. Their objectives were to study the emergence and clearance of gametocytes in Thai patients admitted at the Bangkok Hospital for Tropical Diseases (Piyaphanee et al., 2006). Smalley and Sinden was studied the longevity and infectivity of the *P. falciparum* gametocytes in the West Africa they could found the half-life of the mature gametocytes in the blood was 2.4 days (Smalley and Sinden, 1977). Eichner *et al.* has found the half life of gametocytes was 6.4 days (Range 1.3-22.2 days) (Eichner et al., 2001). Bousema *et al.* has been found the mean duration of gametocytemia in Kenyan children below five year was 9.4, 7.8 day in children between five to nine and 4.1 days in older than ten year. Their main objective was to study the factors that influence gametocytemia in asymptomatic children in the absence and presence of sulphadoxine-pyrimethamine (SP) antimalarial treatment (Bousema et al., 2004). Some gametocytes have been found to have a longevity up to 42 days in the blood stream (Suputtamongkol et al., 2003).

The prevalence of gametocytemia during severe *P.falciparum* has not been well defined. Because of the importance of gametocytes in malaria transmission, high mortality of severe malaria and according to our knowledge there is very limited information regarding gametocytes clearance in complicated malaria so we propose to study the prevalence of gametocytemia, gametocytes emergence and clearance in severe *P.falciparum* malaria.

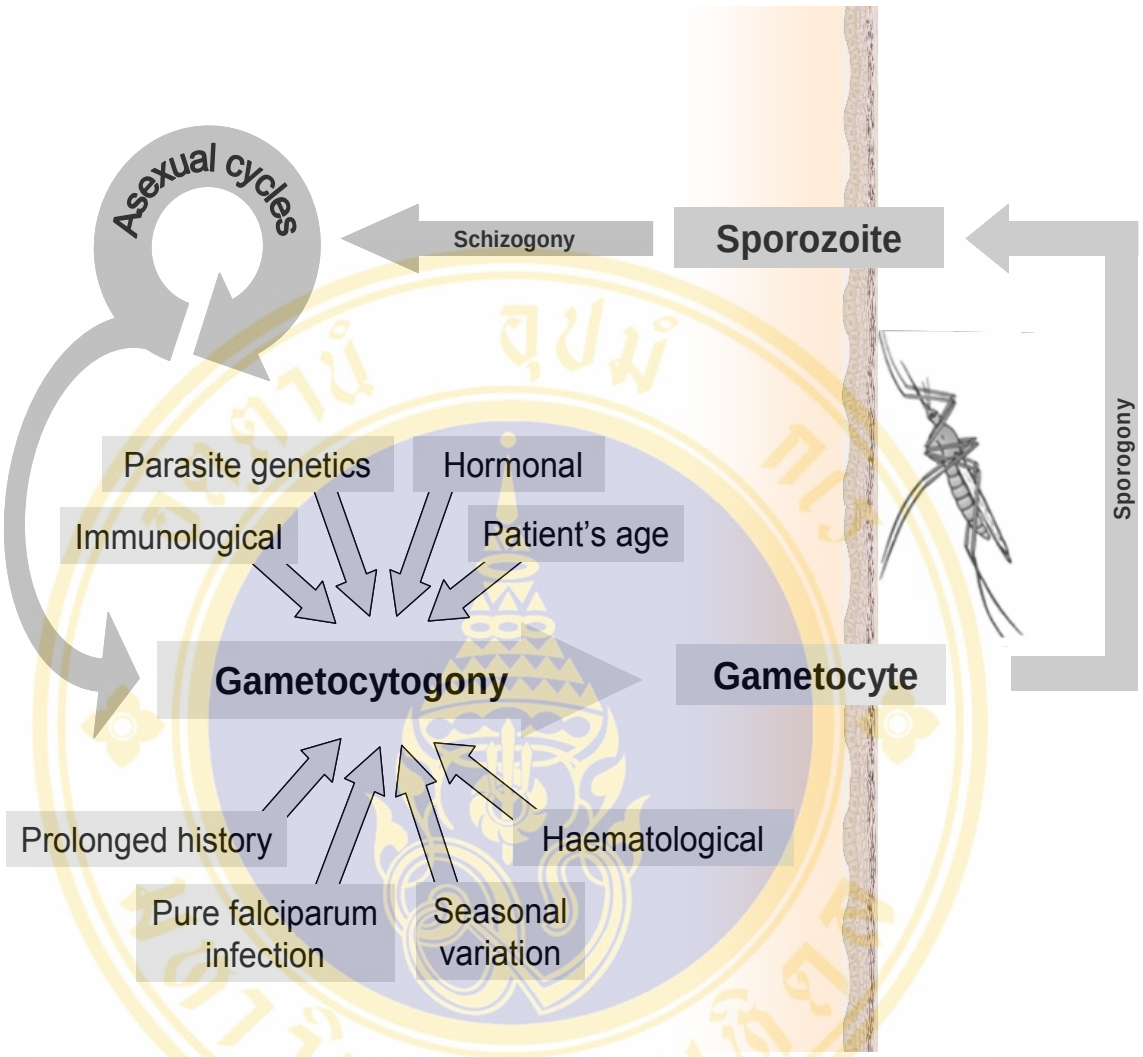

Figure 1: Gametocytes in malaria life cycle and some of the factors affecting gametocytogenesis.

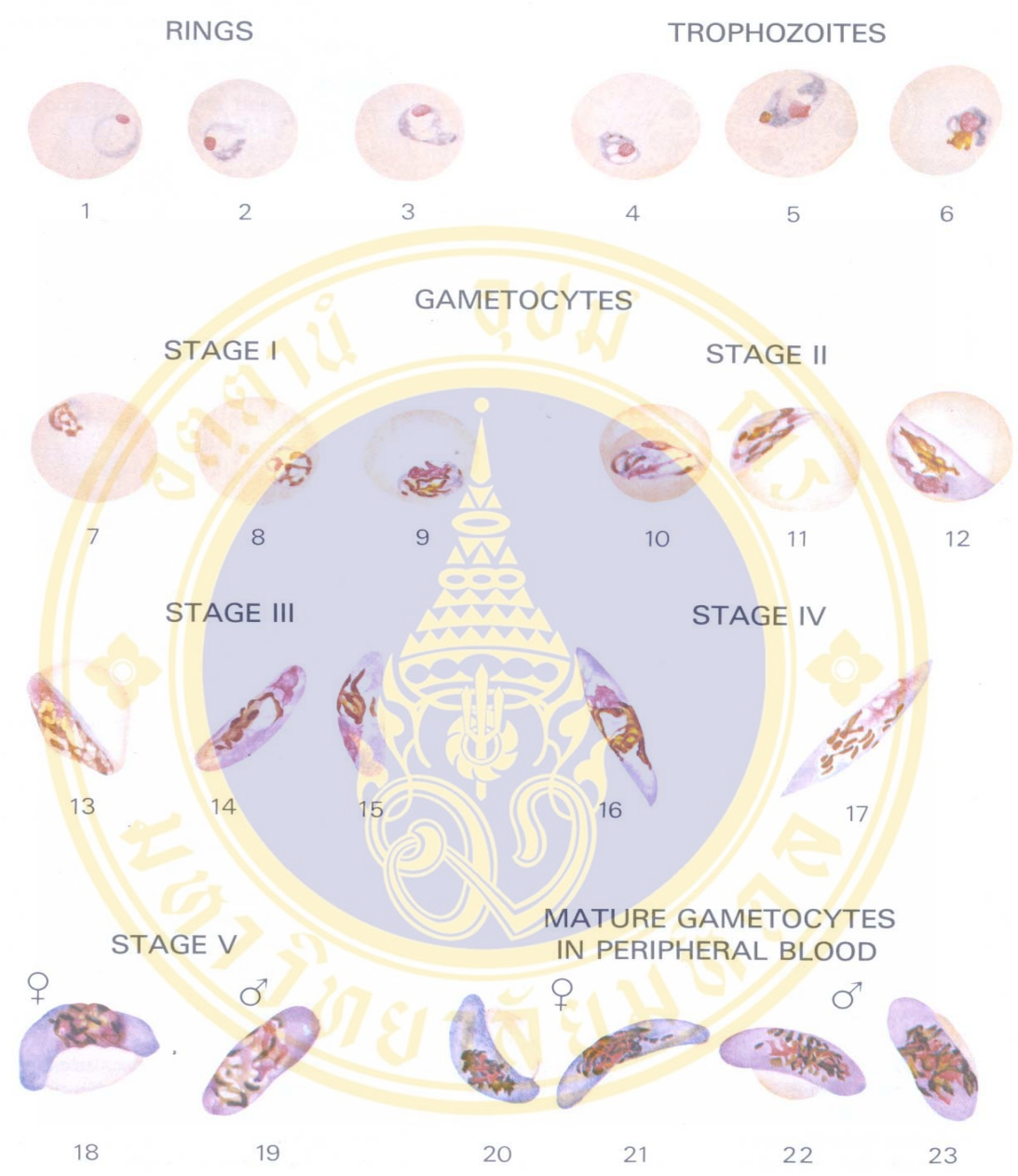

Figure 2: Different stages of *P. falciparum* (W.H.Wernsdorfer Sir I.McGregor, 1988)

CHAPTER IV
MATERIAL AND METHODS

Study site and study design

This retrospective study was conducted in the Bangkok Hospital for Tropical Diseases between November 12, 2007 and January 5, 2008.

Study population

Patients with severe *P. falciparum* malaria admitted at the Bangkok Hospital for Tropical Diseases from January 2000 to December 2006.

Inclusion criteria

- All patients with peripheral blood picture showed ring stage of *P. falciparum.*
- Have had symptoms or signs of severe falciparum malaria according to the criteria of WHO 2006 for severe malaria.
- Had received artesunate as a therapy for severe malaria

Exclusion criteria

- Patients having been received primaquine during admission.
- Patients with missing important data in their records .i.e. results of thick and thin blood film in the first day of admission, initial parasite count, initial gametocyte count.

Diagnosis of malaria

Parasitological diagnosis of malaria had been made by thick and thin blood film for asexual blood stage parasite and gametocyte every 6 hours until asexual

blood stage were no longer present and then daily thereafter until day 28 or until discharge.

Parasite and gametocyte counts per microliter of blood were calculated per 200 white blood cells in the thick blood film or per 1000 red blood cell in thin blood film according to the formula (Monica Cheesbrough, 1981):
In thin blood film parasite or gametocyte per microliter:

$$\text{Parasite Count x RBC } (10^6/\mu l) \text{ x } 1000$$
$$\text{Gametocyte Count x RBC } (10^6/\mu l) \text{ x } 1000$$

In thick blood film parasites or gametocytes per microliter:

$$\frac{\text{Parasite Count x WBC } (10^3/\mu l) \text{ x } 1000}{200}$$

$$\frac{\text{Gametocyte Count x WBC } (10^3/\mu l) \text{ x } 1000}{200}$$

Treatment

All patients with severe *P. falciparum* malaria in this study have received standard antimalarial medication i.e. artemisinin based combination therapy (ACT).

Sample size

The sample size was calculated according to the prevalence of uncomplicated falciparum malaria at the Bangkok Hospital for Tropical Diseases according to the formula below:

$$n = \frac{(Z_{\alpha/2})^2 \, p \, (1 - p)}{\delta^2}$$

The prevalence of gametocytemia in complicated *P. falciparum* is unknown. Therefore we use the data obtained from uncomplicated *P.falciparum* malaria (20%) (Piyaphanee et al., 2006). Therefore: $p = 0.2$

We set the power = 95% and $\alpha = 0.05$, that sets $Z_{\alpha/2} = 1.96$. If $\alpha = 0.05$ and $\delta = 0.05^2$

$$n = \frac{(1.96)^2 \times (0.2)\ (0.8)}{(0.05)^2}$$

Therefore the sample size should be 245.

In each year, there were approximately 60-70 cases of severe *P.falciparum* malaria admitted at the Bangkok Hospital for Tropical Diseases. Therefore, in our study timeframe (2000-2006), we would be able to get an adequate sample size.

Data analysis

Statistical analysis was performed using statistical package for the Social Science (SPSS version 11.5). All the *P*.values was expected to be from two tailed test and statistical significance level was set at 0.05. The distribution of the data was assessed for normality using *kolmogorov-smirnov test*. Numerical data in case of normal distribution presented as mean and standard deviation and *student t test* used to compare the means. Numerical data in case of non-normal distribution presented as median and Range, and *Mann-Whitney U test* was used. Categorical data were expressed as number and percentage. Gametocyte clearance times were evaluated by *Kaplan-Meier analysis*.

Ethical considerations

The study proposal was reviewed and approved by the Ethical Committee of the Faculty of Tropical Medicine of Mahidol University, with the reference number of certificate of ethical approval; MUTM 2007-011

Significance of research

By understanding the prevalence of gametocytemia in severe falciparum malaria and the gametocyte emergence and clearance time, it will be of value to the scientific community about malaria control.

Research fund

The research fund was provided by the Faculty of Tropical Medicine.

Operational definitions

- *Hyperparasitemia:*
  Parasite density of more than 5% in non-immune patient

- *Fever clearance time (FCT):*
  Period from the start of treatment until the oral temperature decreased to 37.5°C and remain below this temperature for the next 48 hours.

- *Parasite clearance time (PCT):*
  Interval between starting treatment and the first peripheral blood smear with no demonstrable sexual parasite.

- *Gametocyte clearance time (GCT):*
  Is the interval between the first and last positive smear for gametocytes.

CHAPTER V
RESULTS

During January 2000 and December 2006 there were 531 patients with severe *P. falciparum* malaria were admitted at the Bangkok Hospital for Tropical Diseases we reviewed their charts. Gametocytemia was found in 254 of these 531 patients. So the 254 patients were our population. The prevalence of gametocytemia was found to be 47.8% (254 out of 531), one hundred sixty four patients (64.6%) were male, the median age was 22 years (Range =5-71). Thirty one percent of patients who would finally developed gametocytemia were Mon, 28.7% were Karen, 20.5% were Thai, 18.1% were Burmese and less than 2% were from Lao, Cambodia, Bangladesh, and Nepal (Figure 3). More than three quarter (80.3%) were having first episode of malaria, 13.8% were having second episode of malaria and 6% having more than two episode of malaria during their life. The median duration of fever prior to admission was 5 days (Range = 1-23). The median fever clearance time (FCT) was 68 hours (Range = 4-262). The median parasites clearance time (PCT) was 57 hours (Range = 8-251). All the patients have been classified as severe malaria according to WHO criteria for severe falciparum malaria. One hundred ninety nine patients (78.3%) have had jaundice. More than half of the patients (57.5%) were suffered from hyperparasitaemia; fifty five patients (21.7%) had acute renal failure. Other complications were metabolic acidosis, cerebral malaria, convulsion, pulmonary edema forty two patients (16.5%), thirty nine patients (15.4%), twenty five patients (9.8%), and seven patients (2.8%) respectively (Figure 4). The median initial parasite count was 213,960/µl (Range = 3-2,137,100) and the median initial gametocytes count was 67 /µl (Range = 6-14,280). The characteristics of the 254 patients are summarized in (Table 1).

First detection of gametocytes:

Gametocytes were detected on admission in 164 patients (64.6%). Gametocytemia was found in 42 patients (16.5%) on the 12 hours blood smear and in an additional 15 patients (5.9%) on the blood smear taken 24 hours after admission (Table 2). Therefore, by 24 hours gametocyte had been observed in 87% of all patients who would finally develop gametocytemia. Gametocytes were emerging in the other 33 patients (13%) during treatment. Some patients developed gametocytemia at late time, as late as 348 hours after admission.

Table 1: Characteristics of the patients with gametocytemia (n = 254)

	Median	Minimum	Maximum	Mean	SD
Age (year)	22	5	71		
BMI(kg/m^2)				20.09	3.04
Initial parasite count/µl*	213,960	33	2,137,100	383,463	290,800
PCT(hours)				62.19	26.37
FCT(hours)				73.51	47.33
GCT(hours)	104.5	2	655		
Initial gametocyte count/µl*	67	6	14,280	406.15	1340.027

*Geometric mean.

Clearance of gametocytes:

Gametocytes were clear in 250 patients (98.4%) and only four patients (1.6%) were discharged with gametocytemia. The median gametocyte clearance time (GCT) was 105 hours (Range = 2-655). The cumulative survival of gametocyte by *Kaplan-Meier survival analysis* is shown in (Figure 5). Gametocytes were cleared within 100 hours after admission in less than half of the patients (49%) and within 200 hours

after admission in more than three quarter (76 %) of the patients. the longest time for gametocyte clearance was found to be 655 hours after admission.

Furthermore, to study the correlation between parasite clearance time and gametocyte clearance time by using non-parametric *spearman's rho correlation*. We found that there is no significant correlation between parasite clearance time and gametocyte clearance time ($p = 0.821$) (Figure 6).

All patients in this study were received Artemisinin therapy. All patients were admitted at the hospital and aim to follow for 28 day. 206 patients (81.1%) were successfully recovered. 35 patients (13.8%) were lost to follow-up, 12 patients (4.7%) had recrudescence and one patient (0.4%) died.

Table 2: First appearances of gametocytes

Hours after admission	Number	Percentage %	Cumulative %
0	164	64.6	64.6
12	42	16.5	81.1
24	15	5.9	87.0
36	13	5.1	92.1
48	7	2.8	94.9
60	5	2.0	96.9
72	1	0.4	97.2
84	1	0.4	97.6
96	1	0.4	98.0
120	1	0.4	98.4
156	1	0.4	98.8
204	1	0.4	99.2
216	1	0.4	99.6
348	1	0.4	100.0
Total	254	100	

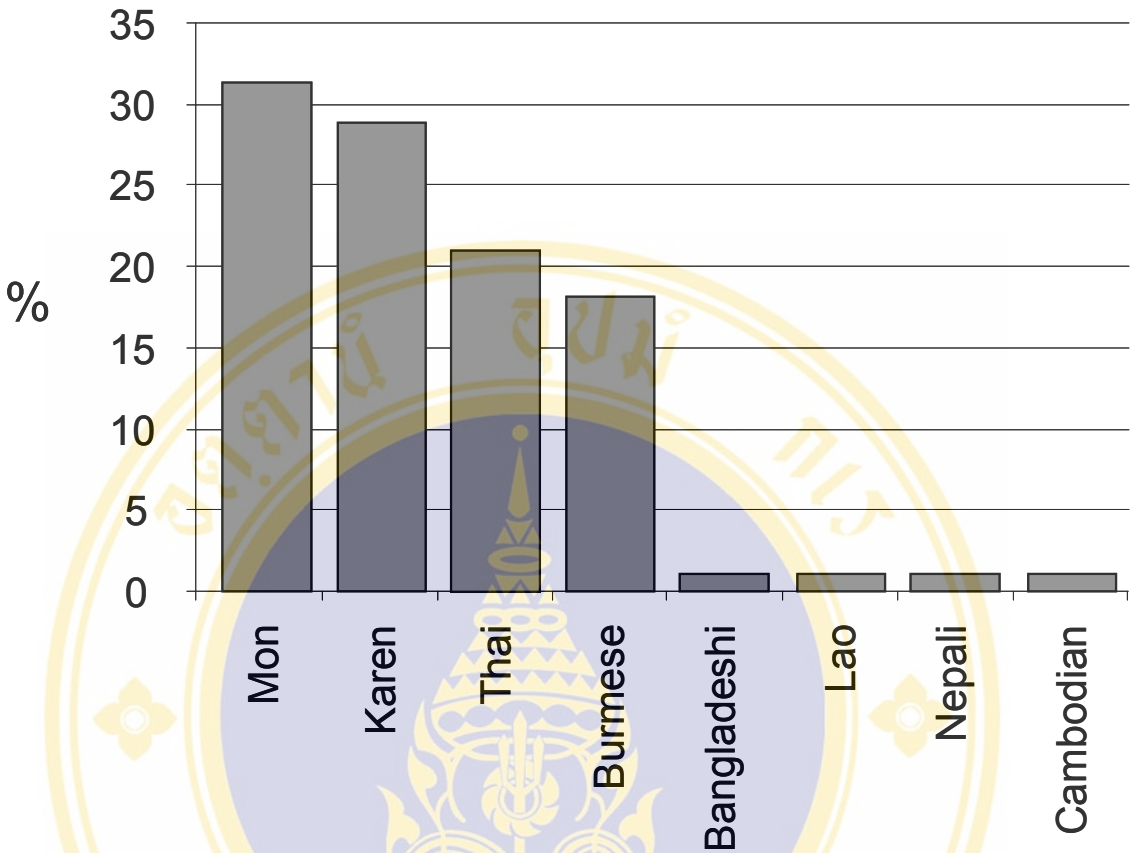

Figure 3: Ethnicity of the patients with gametocytemia.

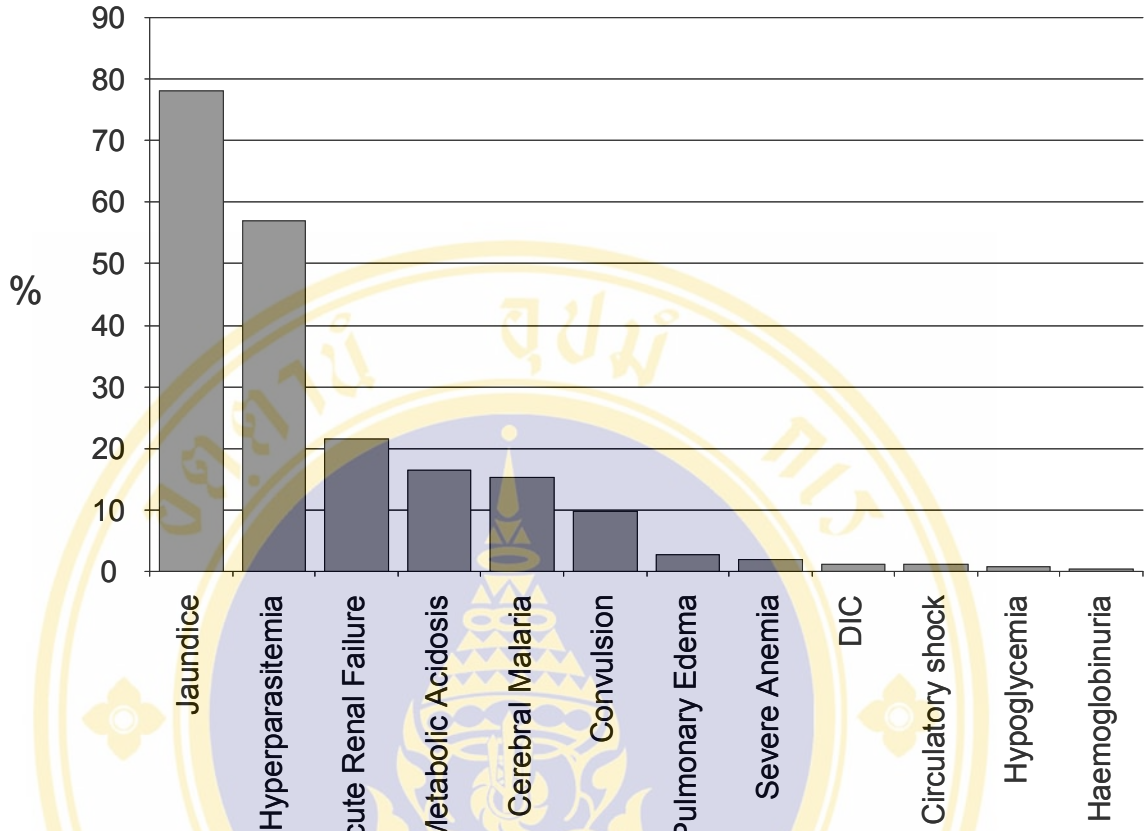

Figure 4: WHO criteria for severe malaria in patients with gametocytemia.

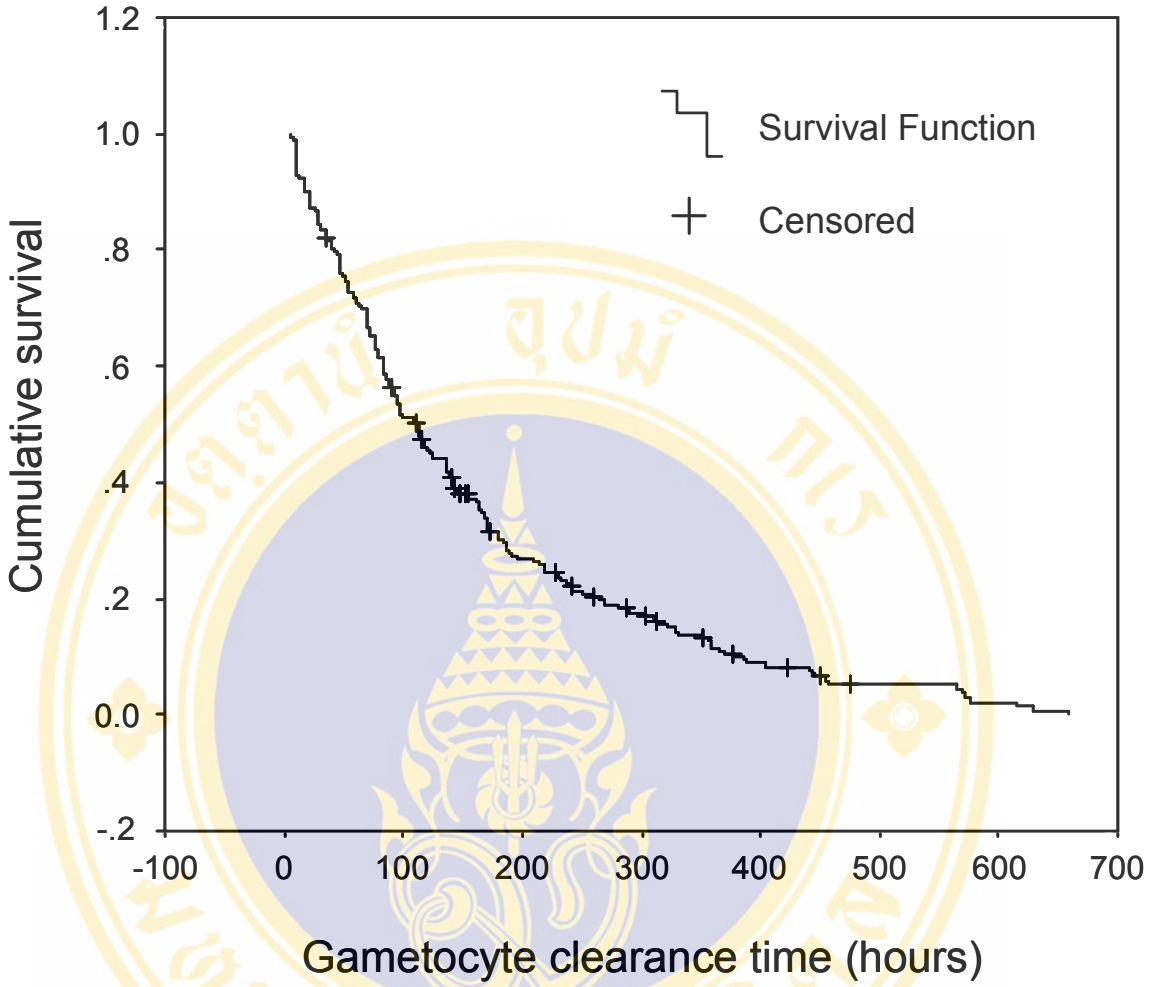

Figure 5: *Kaplan-Meier survival analysis* of gametocyte clearance in the study population.

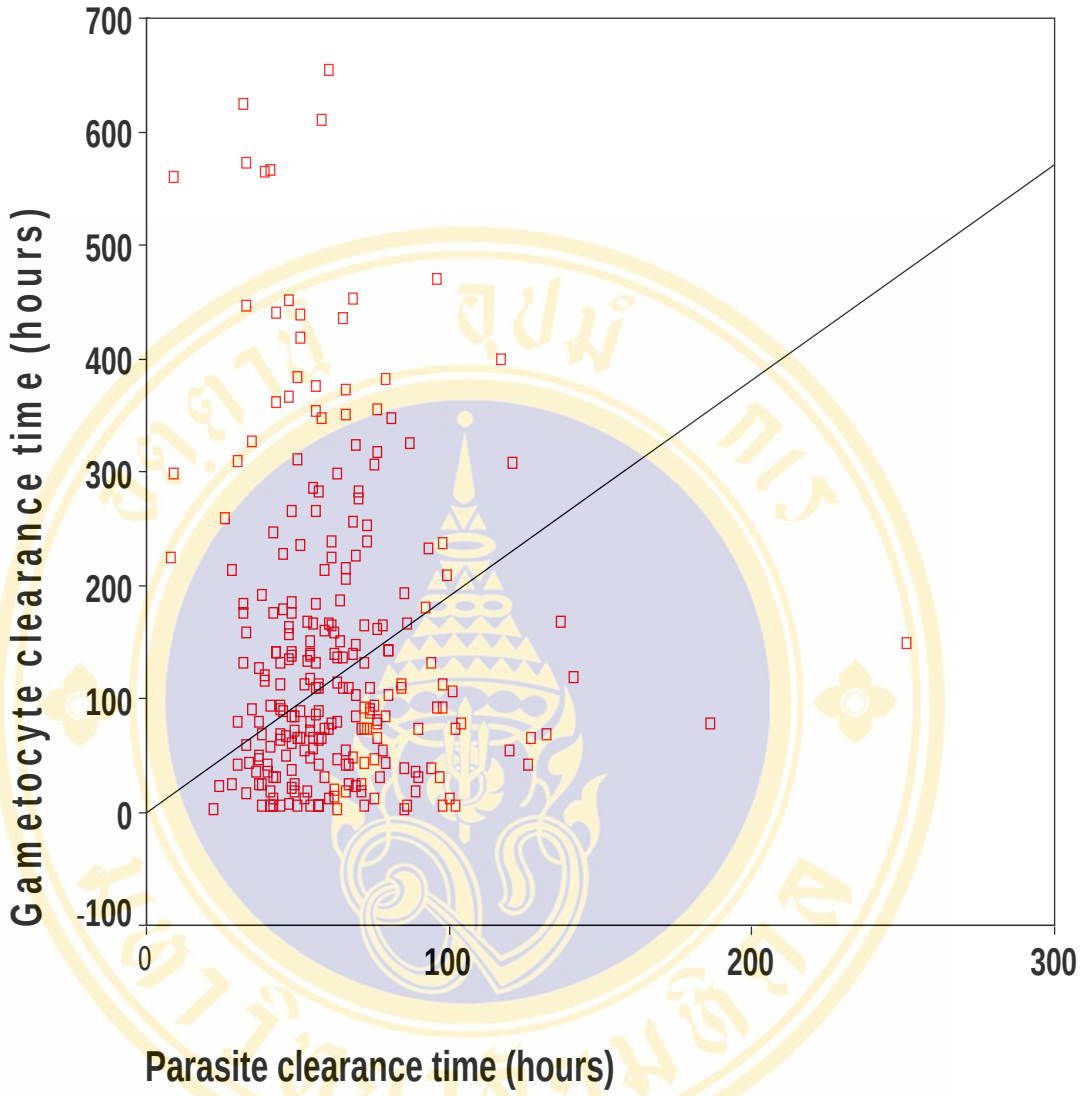

Figure 6: Scatter plot of the correlation between parasite clearance time (hours) and gametocyte clearance time (hours) in patients with gametocytemia

CHAPTER VI
DISCUSSION

Much of the epidemiologic study of the *P. falciparum* focuses on prevalence patterns of asexual parasites in people of different ages, whereas the gametocytes that propagate the disease are often neglected.

The prevalence of gametocytemia during *P.falciparum* malaria has not been well defined although most information comes from outpatient studies. The overall prevalence of gametocytemia in uncomplicated *P.falciparum* has been reported before 2.4-33.8% (Price et al., 1996; Suputtamongkol et al., 2003; Pukrittayakamee et al., 2004; Bousema et al., 2004), The aims of most of these studies was focusing on the efficacy of antimalarial drug on asexual and sexual stage of malaria parasite . The prevalence of gametocytemia has come from point prevalence in most of these studies. Piyaphanee *et al* has been conducted study in 2006; they were focused on the gametocytes. Their objectives were to study prevalence of gametocytemia, gametocyte emergence and clearance time in uncomplicated *P. falciparum* malaria. They could found that the prevalence of gametocytemia was 20.2%. We detect an overall prevalence rate of 47.8% in severe falciparum malaria patients.

Our prevalence result is higher than result by Piyaphanee *et al*. the reasons behind that may be due to severity of malaria or due to long duration of parasitemia (the median PCT was 57 hours vs. 43 hours) or may be due to more gametocytogenesis as the result of prolonged symptoms (median FCT was 68 hours vs. 43 hours). This also indirectly related to the degree of the parasitemia, in our study the median initial parasite count was 212,960 / µl (Range = 33-2,137,100) compare with 8,720 / µl (Range = 17-156,420) in uncomplicated malaria.

In this study the proportion of Mon and Karen ethnic group who developed gametocytemia were very high (31.1%), 28.7% respectively compared with the other ethnic likes Thai (20.5%), Burmese (18.1%), and 0.4% for each Bangladeshi,

Lao, Nepali, Cambodian ethnicity. The high percentages were due to calculating the ratio from the patients who would develop gametocytemia, i.e. not from the population of severe malaria. So the nominator was the numbers of patients with same ethnic group and the dominator were the total number of the patients with gametocytemia. This because we planed to study in detail in those with gametocytemia only, thus we could not know the exact ethnic proportion among the population of severe malaria. So the interpretation of Figure No. 3 should be done with care.

In our study gametocytes appears in the first 24 hours in most of the cases 87%. Our result is similar to those previous studies such as study by Piyaphanee *et al.*, which they found 92.4% of the case have gametocytemia on the first 24 hours. This is might be due to association between prolonged pre-admission history of fever and gametocytogenesis. In both studies the median duration of fever prior to admission was 5 days. The relation between gametocytogenesis and the prolonged pre-admission history of fever was observed in the study by Price *et al.* in 1999. Presence of gametocytes on admission is indirectly indicated that the infection has already occurred before several days.

Gametocyte can be absent on admission and emerge during treatment. In our study the percentage of patients developing gametocytemia if gametocytemia were not present by 24 hours was only 13% (33/254), in Piyaphanee *et al* study was only 7.5% (18/240). This slightly similar result may be due to the sequestrated gametocytes that already in last process of maturation in the organs, it reaches maturity by the time of starting the ACT (which has no effect on mature gametocytes). They were circulated in the peripheral blood and emerge during treatment with artemisinin based combination therapy.

One expected benefit of widespread introduction of artemisinin based combination therapy for malaria is a reduction in gametocyte carriage (Piper et al., 1999), and several studies has been shown that artemisinin derivatives can reduces viability of young sequestrated gametocyte greatly (von Seidlein et al., 1998; Targett et al., 2001; Drakeley et al., 2004) but has little effect on mature gametocyte

(Pukrittayakamee et al., 2004). However in this study 35.4% developed gametocytemia later during treatment with artemisinin therapy.

Primaquine in combination with artesunate has been shown to reduce the gametocyte carriage compare with artesunate alone (Pukrittayakamee et al., 2004). To reduce malaria transmission, current policy by the national program in malaria endemic area of Thailand is to administer artesunate plus mefloquine plus Primaquine to all newly diagnosed *P.falciparum* malaria patients whether gametocytemia is present or not. If we used the same strategy in our study patients would have covered the 47.8% of individual with severe *P.falciparum* malaria. However, primaquine would have been given without indications to the other 52.2% of the patients and exposed them to the risk of G6PD deficiency related hemolysis if they were suffered from it.

Current WHO recommendation to give single, 45 mg dose of primaquine which was effective for clearing gametocytes from the blood, however, one study show that more than 9% of those with the severe falciparum malaria treated with quinine continued to harbour gametocytes in their peripheral blood 15 days after taking the primaquine (Kamtekar et al., 2004). Same as in the Suputtamongkol *et al* study in 2003, in which they observed failure of gametocytemia eradication by combination of mefloquine plus primaquine. So the activity of primaquine may be depends on the choices of schizonticidal drug that used (El Sayed et al., 2007).

Gametocytemia persisted even with single dose of primaquine 45 mg given in the fourth day of admission (Gogtay et al., 2006), in mentioned study the gametocyte remain viable until day 8 of admission in 65% of patients in this study and became negative later. In practice, based on this study and our finding regarding eight patients (3%) developed gametocytemia between day 3 and day15 from admission, the study by Kamtekar *et al* giving primaquine in the first days of admission (1-4 days) with half life of 3 to 6 hours, might not be effective to eradicate the gametocytemia, so the question is when to give primaquine to the patient with or without gametocytemia? The answer of this question needs further investigation.

Many studies found that artemisinin can inhibit gametocytogenesis when compared with quinine (Pukrittayakamee et al., 2004), primaquine can shorten gametocyte clearance time, but even without primaquine, most patients with gametocytemia will eventually clear the gametocytes from their blood. In our study almost all the patients (250/254, 98.4%) who developed gametocytemia can eradicate gametocyte from their blood. Only four (4/254, 1.6%) patients discharged with gametocytemia. This result was similar to those of the study by Piyaphanee *et al* in uncomplicated falciparum malaria in which gametocytemia were cleared in the most of the patients (219/240, 91%) and only (21/240, 9%) were discharge with gametocytemia. These two studies used artemisinin based combination therapy in their patients, and those who were not cleared the parasitemia were lost to follow before completion 28 days.

Previous studies such as Bousema *et al* in 2004 in western Kenya had been shown that the mean duration of gametocytemia was between 4.1 day and 9.4 days. Their study was aimed to identify the factors that influence gametocytemia in asymptomatic children in the absence and presence of sulphadoxine-pyrimethamine (SP) antimalarial treatment. Smalley and Sinden in 1977 in West Africa were aimed to study the longevity and infectivity of the *P. falciparum* gametocytes in patients receiving chloroquine. They could found the half life of the gametocytes was 2.4 days and the longest period for gametocytes was 24 days. In our study we found the median gametocyte clearance time of 105 hours (Range = 2-655). In the other study by Piyaphanee *et al* in 2006 in Thailand in uncomplicated falciparum malaria in which the median gametocyte clearance time was 163 hours (Range = 12-806).

The shorter time of GCT in our study may be due to the some factors i.e. severity of the malaria, or due to due to the action of the antimalarial drug (ACT) on the premature stages of the gametocytes, or the rapid clearance of the asexual stage of the parasite by the ACT (compare with SP, chloroquine that used in the other studies). Or may be due to the duration of the treatment (7 days) in complicated malaria compared with (3 days) in uncomplicated malaria. Or the administration route of the antimalarial drugs that used in complicated and uncomplicated malaria i.e.

intravenous route compared with oral route in which the absorption of the drugs might be alter. Other explanation may be related to the degree of the immunity of our study population compared with other subjects in different studies mentioned above.

Whether to treat all severe *P. falciparum* malaria patients with combination of primaquine with artemisinin derivatives remains controversial, and not all patients with complicated falciparum malaria will develop gametocytemia, exposing patients to the risk of primaquine depend on measuring risk benefit ratio.

In theory, there were positive correlation between asexual parasitemia and gametocytemia in falciparum malaria (Akim et al., 2000). Such relation can be reflected by the correlation between PCT and GCT. Prolong PCT as the result of inappropriate or ineffective treatment can lead to increase the population of a sexual parasite from which fraction would take gametocytes pathway. Thus enhancing gametocytogenesis and eventually prolong GCT. Previous studies have been shown that sexual stage specific immunity during natural infection is regulated by immunity to the asexual stage (McGregor, 1987). Such immunity would clear or greatly reduce the pool of asexual parasite from which gametocyte are derived, so the high asexual parasite density induce more effective immunity to clear asexual parasite leaving few to survival to produce gametocyte.

Same process mentioned above occur with using artemisinin derivatives in the treatment in which fast clearance of asexual parasite leads to small number of gametocyte that finally cleared by the immunity. Although positive correlation between PCT and GCT was considered by using this two concept role of the immunity and artemisinin therapy in clearing the parasite and gametocyte, in our study we found that there was no association between PCT and GCT. This may be due to the severity of malaria, or may be related to our methodology or not enough sample size or early administration of effective treatment ($p = 0.821$).

In present study the mortality rate was less than 1% and the reason behind that might be due to exclusion of the other cases of severe malaria not corresponding to

our inclusion criteria or might be due to the proper case management, proper care facilities in the study site.

Ones of the limitations of our study were its retrospective nature, lack of detection of the viability of the parasite and the gametocytes in the follow up period to know the exact time of parasite and gametocyte clearance with treatment.

To date reported attempts to fight the parasite burden by targeting the gametocyte are commonly overlooked. However, when one considers malaria control strategies as a whole, gametocytes are often secondary targets. Any anti-Anopheline interventions, as reduces vector density, also decrease the passage of gametocytes from man to mosquito. Newly developed drugs, such as combination therapy with artemisinin derivatives, have as their main purpose the efficient removal of asexual stages, but the gametocytocidal activity (against immature form of gametocytes) of those drugs should be considered an important issue.

Gametocytes may play role on spread of antimalarial drug resistant gene. There is a recent concept among the scientific community that control of anti-malarial drug resistance needs involve gametocyte control. Determining the prevalence of gametocytemia and the gametocyte clearance and the effect of different antimalarial drug on gametocyte clearance will provide information for strategy makers to control malaria transmission.

CHAPTER VII
CONCLUSION

Much of the epidemiologic study of the *P. falciparum* focuses on prevalence patterns of asexual parasites in people of different ages, whereas the gametocytes that propagate the disease are often neglected.

We conclude that prevalence of gametocytemia in severe *P.falciparum* malaria in the Bangkok Hospital for Tropical Diseases was 47.8%, in most of cases circulating gametocyte were observed in 87% during the first 24 hours after admission, but also it can emerge during treatment with artemisinin based combination therapy in 13% and the longest interval between admission and detection of the parasite was 15 days.

Not all patients with severe *P. falciparum* malaria will develop gametocytemia and the gametocytes were cleared from the circulation after median time of 105 hours with treatment with artemisinin based combination therapy (ACT), however even with treatment four patients (1.6%) discharged with their gametocytemia.

The higher prevalence of gametocytemia in severe malaria compared with uncomplicated malaria as due to prolongation of symptoms. It might increases malaria transmission in the settings where malaria control program was declining.

Our result concerning the correlation between the parasites clearance time (PCT) and the gametocytes clearance time (GCT) showed no significant correlation. This result was against the previous results which were showed positive correlation between PCT and GCT.

The uses of Primaquine or not, does not affect the transmission of malaria in the settings where malaria vector not existing i.e. Bangkok. If the decision is to give

primaquine to the patients with or without gametocytemia that were treated in bangkok, further investigation is needed to answer the question when the best time to give primaquine to the patients?

The early and effective administration of artemisinin based combination therapy (ACT) to severe malaria patients can interrupt malaria transmission by indirect action through its fast clearance of asexual stage of the parasites. So the role of these drugs as gametocytocidal drug should be considered an important issue.

Gametocytes may play role on spread of antimalarial drug resistant gene. Control of anti-malarial drug resistance needs involve gametocyte control.

Information regarding prevalence of gametocytemia and the effects of different currently used antimalarial drugs on the prevalence will help the policy makers to decide effective strategy for malaria prevention.

REFERENCES

Akim NI, Drakeley C, Kingo T, Simon B, Senkoro K, and Sauerwein RW. Dynamics of P. falciparum gametocytemia in symptomatic patients in an area of intense perennial transmission in Tanzania. Am J Trop Med Hyg 2000;63:199-203.

Alano P and Carter R. Sexual differentiation in malaria parasites. Annu Rev Microbiol 1990;44:429-449.

Bousema JT, Gouagna LC, Drakeley CJ et al. Plasmodium falciparum gametocyte carriage in asymptomatic children in western Kenya. Malar J 2004;3:18.

Bruce MC, Alano P, Duthie S, and Carter R. Commitment of the malaria parasite Plasmodium falciparum to sexual and asexual development. Parasitology 1990;100 Pt 2:191-200.

Chotivanich K, Sattabongkot J, Udomsangpetch R et al. Transmission-blocking activities of quinine, primaquine, and artesunate. Antimicrob Agents Chemother 2006;50:1927-1930.

Collins WE and Jeffery GM. A retrospective examination of sporozoite- and trophozoite-induced infections with Plasmodium falciparum in patients previously infected with heterologous species of Plasmodium: effect on development of parasitologic and clinical immunity. Am J Trop Med Hyg 1999;61:36-43.

David A.Warrell and Herbert M.Gilles. Essential Malariology. 4. London: David A.Warrell and Herbert M.Gilles; 2002.

Dondorp AM, Kager PA, Vreeken J, and White NJ. Abnormal blood flow and red blood cell deformability in severe malaria. Parasitol Today 2000;16:228-232.

Dondorp AM, Pongponratn E, and White NJ. Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology. Act Trop 2004;89:309-317.

Drakeley C, Sutherland C, Bousema JT, Sauerwein RW, and Targett GA. The epidemiology of Plasmodium falciparum gametocytes: weapons of mass dispersion. Trends Parasitol 2006;22:424-430.

Drakeley CJ, Jawara M, Targett GA et al. Addition of artesunate to chloroquine for treatment of Plasmodium falciparum malaria in Gambian children causes a significant but short-lived reduction in infectiousness for mosquitoes. Trop Med Int Health 2004;9:53-61.

Drakeley CJ, Secka I, Correa S, Greenwood BM, and Targett GA. Host haematological factors influencing the transmission of Plasmodium falciparum gametocytes to Anopheles gambiae s.s. mosquitoes. Trop Med Int Health 1999;4:131-138.

Dyer M and Day KP. Commitment to gametocytogenesis in Plasmodium falciparum. Parasitol Today 2000;16:102-107.

Eichner M, Diebner HH, Molineaux L, Collins WE, Jeffery GM, and Dietz K. Genesis, sequestration and survival of Plasmodium falciparum gametocytes: parameter estimates from fitting a model to malariatherapy data. Trans R Soc Trop Med Hyg 2001;95:497-501.

El Sayed B, El Zaki SE, Babiker H et al. A Randomized Open-Label Trial of Artesunate- Sulfadoxine-Pyrimethamine with or without Primaquine for Elimination of Sub-Microscopic P. falciparum Parasitaemia and Gametocyte Carriage in Eastern Sudan. PLoS ONE 2007;2:e1311.

Franco-Paredes C and Santos-Preciado JI. Problem pathogens: prevention of malaria in travellers. Lancet Infect Dis 2006;6:139-149.

Gogtay NJ, Kamtekar KD, Dalvi SS et al. A randomized, parallel study of the safety and efficacy of 45 mg primaquine versus 75 mg bulaquine as gametocytocidal agents in adults with blood schizonticide-responsive uncomplicated falciparum malaria [ISCRTN50134587]. BMC Infect Dis 2006;6:16.

Graves PM, Carter R, and McNeill KM. Gametocyte production in cloned lines of Plasmodium falciparum. Am J Trop Med Hyg 1984;33:1045-1050.

Kamtekar KD, Gogtay NJ, Dalvi SS et al. A prospective study evaluating the efficacy of a single, 45-mg dose of primaquine, as a gametocytocidal agent, in patients with Plasmodium falciparum malaria in Mumbai, India. Ann Trop Med Parasitol 2004;98:453-458.

Lingnau A, Margos G, Maier WA, and Seitz HM. The effects of hormones on the gametocytogenesis of Plasmodium falciparum in vitro. Appl Parasitol 1993;34:153-160.

Lo RV, III and Gluckman SJ. Prevention of malaria in travelers. Am Fam Physician 2003;68:509-514.

MacPherson GG, Warrell MJ, White NJ, Looareesuwan S, and Warrell DA. Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. Am J Pathol 1985;119:385-401.

McGregor IA. Malarial immunity: current trends and prospects. Ann Trop Med Parasitol 1987;81:647-656.

Monica Cheesbrough. Medical Laboratory Manual for Tropical Countries. First. hertford: 1981.

Nacher M, Carrara VI, Ashley E et al. Seasonal variation in hyperparasitaemia and gametocyte carriage in patients with Plasmodium falciparum malaria on the Thai-Burmese border. Trans R Soc Trop Med Hyg 2004;98:322-328.

Pasvol G. The treatment of complicated and severe malaria. Br Med Bull 2006;75-76:29-47.

Piper KP, Hayward RE, Cox MJ, and Day KP. Malaria transmission and naturally acquired immunity to PfEMP-1. Infect Immun 1999;67:6369-6374.

Piyaphanee W, Krudsood S, Tangpukdee N et al. Emergence and clearance of gametocytes in uncomplicated Plasmodium falciparum malaria. Am J Trop Med Hyg 2006;74:432-435.

Price R, Nosten F, Simpson JA et al. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. Am J Trop Med Hyg 1999;60:1019-1023.

Price RN, Nosten F, Luxemburger C et al. Effects of artemisinin derivatives on malaria transmissibility. Lancet 1996;347:1654-1658.

Pukrittayakamee S, Chotivanich K, Chantra A, Clemens R, Looareesuwan S, and White NJ. Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria. Antimicrob Agents Chemother 2004;48:1329-1334.

Silvestrini F, Alano P, and Williams JL. Commitment to the production of male and female gametocytes in the human malaria parasite Plasmodium falciparum. Parasitology 2000;121 Pt 5:465-471.

Singh N, Chand SK, Mishra AK et al. Epidemiology of malaria in an area of low transmission in central India. Am J Trop Med Hyg 2006;75:812-816.

Smalley ME and Sinden RE. Plasmodium falciparum gametocytes: their longevity and infectivity. Parasitology 1977;74:1-8.

Smith TG, Lourenco P, Carter R, Walliker D, and Ranford-Cartwright LC. Commitment to sexual differentiation in the human malaria parasite, Plasmodium falciparum. Parasitology 2000;121 (Pt 2):127-133.

Suputtamongkol Y, Chindarat S, Silpasakorn S et al. The efficacy of combined mefloquine-artesunate versus mefloquine-primaquine on subsequent development of Plasmodium falciparum gametocytemia. Am J Trop Med Hyg 2003;68:620-623.

Talman AM, Domarle O, McKenzie FE, Ariey F, and Robert V. Gametocytogenesis: the puberty of Plasmodium falciparum. Malar J 2004;3:24.

Targett G, Drakeley C, Jawara M et al. Artesunate reduces but does not prevent posttreatment transmission of Plasmodium falciparum to Anopheles gambiae. J Infect Dis 2001;183:1254-1259.

von Seidlein L, Bojang K, Jones P et al. A randomized controlled trial of artemether/benflumetol, a new antimalarial and pyrimethamine/sulfadoxine in the treatment of uncomplicated falciparum malaria in African children. Am J Trop Med Hyg 1998;58:638-644.

W.H.Wernsdorfer Sir I.McGregor. Principles and Practice of Malariology. London: 1988.

White NJ. Malaria. In : Gordon CC and Zumla A eds. Manson - Tropical Diseases. 21st. W.B. Saunders; 2007 p. 1205-1295.

WHO. World Malaria Report 2005. Geneva, Switzerland: 2005.

WHO. Guidelines for the treatment of malaria. Switzerland: 2006a.

WHO. Regional guideline for the management of severe falciparum malaria in large Hospitals. 2006b.

APPENDIX

APPENDIX

DATA COLLECTION FORM

Patient data

- Patient code ……/……/……
- Date of admission …../……./…… Time ……..
- Age ……..years
- Gender ☐ male ☐ female (Male =1:female = 0)
- Weight ……. kg
- Height …… cm
- Ethnicity ……
- Occupation ……..

History

- History of previous malaria infection ……..
- Days of fever prior to admission ……Days

Physical examination

- Temperature …… C
- Respiratory rate …… min
- Pulse rate …… min
- Blood pressure ……mm/ Hg
- Anemia ☐1 ☐ 0 ☐ 9
- Jaundice ☐1 ☐ 0 ☐ 9
- Dehydration ☐1 ☐ 0 ☐ 9
- Altered consciousness ☐1 ☐ 0 ☐ 9

1=Yes 0=No =9 No data

Complication

- Cerebral malaria ☐1 ☐ 0 ☐ 9
- Pulmonary edema ☐1 ☐ 0 ☐ 9
- Acute renal failure ☐1 ☐ 0 ☐ 9

1= serum Cr ≥265 mmol/l (3.0mg/dl). 0 = serum Cr <265 mmol/l (3.0mg/dl)

- Hypoglycemia ☐1 ☐ 0 ☐ 9

1 = Glucose ≤ 2.2 mmol/l (40mg/dl). 0 = glucose > 2.2 mmol/l (40mg/dl)

- Hyperparasitemia ☐1 ☐ 0 ☐ 9

1 = parasite density ≥5%. 0= parasite density <5 %

- Repeated generalized convulsion ☐1 ☐ 0 ☐ 9

1 = more than 2 /24hours 0 = less than 2 /24hours

- Circulatory collapse (shock) ☐1 ☐ 0 ☐ 9

1 = systolic BP ≤ 70 mmHg+ sign of shock. 0 = systolic BP > 70 mmHg

- Spontaneous bleeding /DIC ☐1 ☐ 0 ☐ 9
- Acidosis ☐1 ☐ 0 ☐ 9

1 =Serum HCO3≤15mmol/l. 0 = Serum HCO3>15mmol/l

- Severe anemia ☐1 ☐ 0 ☐ 9

1=Hb≤5g/dl 0=Hb>5g/dl 9= No data

- Hemoglobinuria ☐1 ☐ 0 ☐

1=Yes 0=No =9 No data

- Jaundice ☐1 ☐ 0 ☐ 9

1=Serum bilirubin ≥3mg/dl. 0= Serum bilirubin <3mg/dl

Investigation

RBC……..……WBC…….…….Platelet count……

Initial parasite count……..……. µl

Time of the gametocyte appearance (GET)…….. hours

Initial gametocyte count…….…… µl

Gametocyte clearance time (GCT)…… hours

Fever clearance time (FCT)…….... hours

Parasite clearance time (PCT)..……... hours

Treatment given ☐ i.v. artesunate + mefloquine

☐ i.v. artesunate + tetracycline /doxycycline

☐ i.m. artemether

☐ Other treatment specify……..

Outcome 1 ☐ Recover

2 ☐ Death

0 ☐ loss of follow up

9 ☐ No data

3 ☐ Recrudescence

BIOGRAPHY

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