CLINICAL MANIFESTATION OF UNCOMPLICATED FALCIPARUM MALARIA AND VIVAX MALARIA IN THAI CHILDREN

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ABSTRACT

This retrospective study was conducted with 170 uncomplicated falciparum and vivax malaria patients. The data were from pediatric patients admitted to the Hospital for Tropical Diseases or Thongpaphum Hospital, from 1991 to 2003. The objective of the study was to compare clinical manifestations, laboratory findings and outcomes of treatment. There were 77 patients with vivax malaria, 90 with falciparum malaria and 3 with mixed infection.

Weakness and dehydration were significantly higher in falciparum malaria, but chills were more prevalent in vivax malaria.

In laboratory findings, white blood cell count, eosinophil, basophil, lymphocyte, abnormal lymphocyte, monocyte, and sodium levels of falciparum malaria were significantly lower than vivax malaria.

All 77 patients with *P. vivax* malaria received a standard chloroquine regimen. The mean fever clearance time (FCT) was 36.7 hours and the mean parasite clearance time (PCT) was 58.6 hours. The cure rate was 100% and neither recrudescence nor relapse was observed during the follow-up period. There was no difference in outcome according to time sequence.

Fifty-four falciparum malaria cases received oral quinine 10 mg/kg every 8 hours for 4 days, and then 15 mg base/kg every 8 hours for 4 days. The mean FCT and PCT were 66 and 68 hours, respectively. The cure rate was 75.4%. The remaining falciparum malaria (31 patients) was treated with rectal artesunate 10-19 mg/kg once a day, followed by mefloquine 25 mg base/kg in two divided doses. The mean FCT and PCT were 39 and 50 hours, respectively. The cure rate was 91.6%. Only 2 patients had reappearance of asexual *P. falciparum* in their circulation, on day 20.

In conclusion, clinical features and laboratory findings seem unhelpful for differentiating the cause of uncomplicated malaria. Chloroquine treatment of vivax malaria was still effective.

KEY WORDS: UNCOMPLICATED MALARIA, CHILDREN, CLINICAL MANIFESTATIONS

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LIST OF ABBREVIATIONS

Abbreviation or symbol	Term
Alk phosphatase	alkaline phosphatase
ALT	alanine aminotranferase
AST	aspartate aminotranferase
ºC	degree Celsius
d	day (s)
DIC	disseminated intravascular coagulation
dL	deciliter
e.g.	for example
et al.	and others
etc	and the others
FCT	fever clearance time
ºF	degree Fahrenheit
g	gram
G-6-PD	glucose-6-phosphate dehydrogenase
h	hours
Hb	hemoglobin
HCO_3	bicarbonate
i.e.	that is
kg	kilogram
L	liter
mEq	miliequivalent
mg	miligram
min	minute
mmHg	millimeter mercury
N	number

LIST OF ABBREVIATIONS (cont.)

Abbreviation or symbol	**Term**
PCT	parasite clearance time
P. falciparum	*Plasmodium falciparum*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*
Q	quinine
SD	standard deviation
T	tetracycline
Th	T helper lymphocyte
U	unit
WHO	world health organization
µL	microliter
%	percentage

CHAPTER I

INTRODUCTION

Malaria is a common and well known disease in tropical countries. It still remains one of the major health problems in these areas and is associated with significant morbidity and mortality. Mostly, adults and children living in malaria endemic areas or people travel to those places are at increased risk in contracting the disease. Several strategies have been used in an attempt to suppress and control malaria including the use of appropriate clothing, netting, insect repellent bed nets (Hill, 1988).

In general, the clinical symptoms of malaria are caused by the development of asexual parasites in infected erythrocytes (English et al., 1996). In *Plasmodium falciparum* (*P. falciparum*) malaria, paroxysm of fever is not common. Continuous fever and irregular anemia are more common and more marked in children than in adults. Severe malaria in adults and children exhibit a different pattern of symptoms (Clark et al., 1997). Both groups have prostration and impaired consciousness, whereas respiratory distress (i.e. acidotic breathing) and convulsion are more commonly found in children with *P. falciparum* infection (Connor et al., 1976). The laboratory findings in children suffering from *P. falciparum* malaria are especially striking, e.g. severe anemia, hypoglycemia, acidosis and hyperlactatemia are predominant features in severe childhood malaria (White et al., 1983).

The treatment of childhood malaria generally comprises of drugs such as chloroquine, mefloquine, quinine and fansidar®. In areas with resistance to the mentioned drugs, either artesunate or arthemeter is used (Looareesuwan et al., 1995). Of those, chloroquine needs special attention because it is safe and inexpensive. Chloroquine had been the choice of treatment for many decades for malaria caused by *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. However, now *P. falciparum* resists to chloroquine in most part of the world. Since *P. vivax* infection is not a fatal disease but *P. falciparum* infection can be a fatal disease. The clinical manifestation and laboratory finding that can differentiate between *P. vivax* and *P. falciparum* are important to make an early diagnosis and proper treatment for preventing complications leading to death from *P. falciparum.* Therefore, we studied on mild clinical manifestations of *P. vivax* and *P. falciparum* infection.

CHAPTER II

OBJECTIVES

Primary Objective:

To study clinical manifestations, laboratory findings and treatment of vivax and uncomplicated falciparum malaria in Thai children.

Secondary Objectives:

1. To compare the clinical presentations between vivax and uncomplicated falciparum malaria.
2. To compare the laboratory findings between vivax and uncomplicated falciparum malaria.
3. To compare the outcome of chloroquine treatment of vivax malaria according to time sequence.
4. To describe the outcome of quinine treatment in *P. falciparum* infection.

CHAPTER III

REVIEW OF LITERATURE

Malaria is a protozoal disease transmitted by the bite from an infected female Anopheles mosquito. It is caused by minute parasitic protozoa of the genus *Plasmodium* that infects human and insect hosts alternatively. It is a very old disease and prehistoric man is thought to have suffered from malaria (WHO, 1992).

The most important event in the history of malaria took place towards the end of the nineteenth century, when the sciences of bacteriology and pathology were discovering the causes of infectious disease, observing the morbid changes in the organs and tissues and also perceiving the role of insects in the transmission of some infection (Karbwang et al., 1992).

PATHOGENESIS

There are four recognized species of malaria parasites of humans, *P. malariae*, *P. vivax*, *P. falciparum*, *P. ovale* (Bradley et al., 1996).

The life cycle of malaria reveals that when sporozoites from salivary glands of the mosquito are inoculated during blood meal into the human blood stream. The organisms invade hepatic parenchymal cells and once initiated, they complete the intrahepatic multiplication. The products of the liver stage (the exoerythrocytic merozoites) are liberated in their thousands from each parenchymal cell to blood stream.

Here they attach to and invade circulating erythrocytes. Inside the erythrocyte, asexual division begins and over a period of 48 hours (h) (*P. falciparum*, *P. vivax*, *P. ovale*) or 72 h (*P. malariae*), the parasites develop through a well-defined series of morphological changes from "ring form" to trophozoites and finally to schizonts containing daughter erythrocytic merozoites. They are liberated by red cell lysis and immediately invade uninfected erythrocytes producing a repetitive cycle of invasion and multiplication (Warrell et al., 1990).

CLINICAL FEATURES

1. Clinical Features of Uncomplicated Falciparum Malaria in Children:

The symptoms include fever, restless, refused food, and may complain of headache and nausea. Infants may present as abdominal distress and older children may exhibit refer pain to the liver or spleen. The liver is often enlarged and slightly tender, likewise the spleen. The stool is often loose and dark green, mucus may be seen in stool but some of them may be constipated (WHO, 1988).

2. Clinical Features of Vivax Malaria in Children:

The primary attack in older children begins with headache, back pain, nausea and general malaise. These prodromal symptoms are mild or absent in relapses. The fever is irregular for 2-4 days(d), but soon become intermittent. At first, there is no regularity in the pattern of fever because several brood of the parasite are not synchronized, but later the 48-hour periodicity becomes predominant chiefly in the afternoon or evening. The temperature may rise to 40.6^{o}C (105^{o} F) or higher. Nausea and vomiting may be severe. The liver and spleen are palpable during the second week of infection (Maegraith, 1977).

Vivax malaria is important not because of its fatality but mainly on account of the debility that it produces as a result of relapses. It is often suppressed by the more virulent *P. falciparum* (Oh, 2001).

3. Clinical Features of Severe Falciparum Malaria in Children:

P. falciparum is responsible for the most severe clinical consequences including cerebral malaria, hypoglycemia, acidosis, anemia, respiratory distress and hyperpyrexia (Warrell et al., 1992).

LABORATORY FINDINGS

Anemia, leukopenia, and thrombocytopenia are usual. The reticulocyte count is normal or decrease. Abnormalities in liver function tests may cause diagnostic confusion with viral hepatitis. Serum transaminases, e.g. alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are usually elevated. Both the direct and the indirect bilirubin levels can elevate (Murphy et al., 1993). Hyponatremia often occurs with *P. falciparum* infection. For vivax malaria, malnourished children with hypoalbuminemia may develop generalized edema during an acute attack of malaria, relapses are common, parasitemia rates are usually lower than in falciparum malaria (Phillips et al., 1985).

TREATMENT OF MALARIA

Treatment depends on the species of the parasite and the geographical areas where resistance are prevalent. Unfortunately, widespread use of chloroquine led to resistance of the parasite especially the deadly falciparum parasite (Bloland et al., 1993). Chloroquine is still used for treating malaria in chloroquine sensitive areas. The dosage of chloroquine is 10 mg base/kg then 5 mg base/kg 6 hours later, and 5 mg base/kg at 24 and 48 hours (Adelusi et al., 1982). In the case of falciparum malaria resistance to chloroquine, quinine sulfate is used in the regimen of 10 mg base/kg 8 hourly for seven days plus tetracycline 5 mg/kg 6 hourly for 7 days given concurrently (Q7T7) for children older than 7 years and quinine sulfate 10 mg base/kg 8 hourly for 4 days then 15-20 mg base/kg 8 hourly for 4 days (Q4-4) for children under 7 years of age (Sabchareon et al., 1988). Mefloquine (Larium®) in the dosage of 15 mg base/kg initially and then 10 mg base/kg 6-12 hours later (total 25 mg base/kg) may be used (Bunnag et al., 1992). At the present in some areas of chloroquine, sulfonamide/pyrimethamine and mefloquine resistance and reduced sensitivity to quinine, oral artesunate or artemether 4 mg/kg/day for 3 days followed by mefloquine 15 mg base/kg initially and then 10 mg base/kg 6-12 hours later are recommended (White, 1985). Vivax malaria should be treated with chloroquine orally, in the dose of 25 mg base/kg given over 3 days. For prevention of relapsed, with has been recommended in case of normal glucose-6-phosphate dehydrogenase (G-6-PD) level, primaquine 0.3 mg/kg/day for 14 days is recommended (Schwartz et al., 2000). In case with G-6-PD deficiency, a weekly dose of 0.9 mg base/kg of primaquine and 5 mg base/kg of chloroquine for a period of 8 weeks has been shown to produce less hemolysis than the 14-day course of primaquine (Dwivedi et al., 2003). For severe and complicated falciparum malaria, the special problems are the management of severe malaria in children and the problem of diagnosis, including the clinical similarities between malaria acidosis and pneumonia, and the difficulty of distinguishing between cerebral malaria and other diseases causing encephalitis or meningitis (Warrell, 1992). Immediate management should be affording for the coma (cerebral malaria) including maintaining airway, and exclude other treatable causes of coma (e.g. hypoglycemia, bacterial meningitis). Tepid sponging, fanning, cooling blanket, and antipyretic are indicated for hyperpyrexia. Anticonvulsant (diazepam or paraldehyde injection) is used when indicated (Hien et al., 1992). Measurement of blood glucose and administration of 50% dextrose intravenously followed by 10% dextrose infusion in case of hypoglycemia is recommended (White, 1992). Fresh whole blood transfusion is indicated in the severe anemia patients. For acute renal failure, it is empirical management to exclude pre-renal causes, check fluid balance, urinary sodium, and give diuretic/dopamine or peritoneal dialysis if urine output is still inadequate despite fluid replacement, (Mehta et al., 2001). We should transfuse screened fresh whole blood in the case of spontaneous bleeding and coagulopathy. In patients who have metabolic acidosis and shock, hypoglycemia should be excluded as well as gram-negative septicemia. Blood culture should be done and parenteral antimicrobials should be given if septicemia is highly suspected. Correction of hemodynamic disturbance and administration of oxygen are also necessary (Migasena, 1983). The choice of treatment regimen is based on local cost and availability of antimalarial drugs (Hall, 1976).

CHAPTER IV

MATERIALS AND METHODS

Study Design:

This was a retrospective descriptive study.

Study Site:

The study was carried out at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok and Thongpaphum Hospital, Kanchanaburi, THAILAND.

Study Period:

The study was conducted from November 2003 to March 2004.

Study Population:

One hundred and seventy pediatric patients with uncomplicated falciparum malaria or vivax malaria admitted to the Hospital for Tropical Diseases, Bangkok or Thongpaphum Hospital, Kanchanaburi province, THAILAND from 1991 to 2003, were enrolled in this study.

Inclusion Criteria:

1. The patient aged less than 15 years old.
2. Admitted with clinical uncomplicated *P. falciparum* and/or *P. vivax* malaria.
3. Laboratory presented asexual stage of *P. falciparum* and/or *P. vivax* in blood smear detected by microscope.
4. Hospital record was available.

Exclusion Criteria:

1. The patients had severe malaria as defined by WHO 2000 (Appendix A).
2. The patients had significant con-commitent disease (e.g.. enteric fever, leptospirosis, pneumonia, etc.).
3. Afebrile during admission time.
4. Immunocompromised host from history.
5. Had history of recent malarial treatment within 1 month or antibiotic treatment (i.e. doxycycline, tetracycline, cotrimoxazole) within 3 days.

Collected parameters:

1) Demographic data
   - Age
   - Sex
   - Height
   - Weight
   - Date of admission
   - Date of illness
   - Date of discharge
   - Previous history of malaria attack
2) Data of clinical manifestations
   - Temperature
   - Blood pressure
   - Pulse rate
   - Respiratory rate
   - Headache
   - Anorexia
   - Nausea
   - Vomiting
   - Diarrhea
   - Cough

- Chills / Rigor
- Myalgia
- Abdominal pain
- Icthing
- Weakness
- Hydration status
- Hepatomegaly
- Splenomegaly

3) Data of laboratory results
- Complete blood count
- Platelet count
- G-6-PD status
- Total bilirubin
- Direct bilirubin
- Alkaline phosphatase (Alk phosphatase)
- AST
- ALT
- Blood glucose
- Serum electrolytes
- Parasite count on admission

4) Regimen of treatment:

5) Outcome of treatment:
- Fever clearance time (FCT)
- Parasite clearance time (PCT)
- Cure/not cure

DATA ANALYSIS

Descriptive statistics was used to describe the distribution of demographic data. Unpaired t-test was used for comparing the continuous variables. Chi-square and Fisher's exact tests were used for qualitative variables. Epi-Info 6.04 and SPSS version 11.0 were used for statistical analysis. Statistical significance in all tests was accepted at two-tailed P value < 0.05.

SAMPLE SIZE CALCULATION

Sample size calculation was performed using the Epi-Info 6.04 calculation. Sample size to compare two proportions was calculated, with predicted proportions of thrombocytopenia of 25% in *P. falciparum* and 8% in *P. vivax* infection (Moulin et al 2003; Echeverri et al 2003).

In order to obtain a confidence level of 90% and power of 80% in detecting the difference of proportion, 85 children in each group or total 170 children were recruited.

RESEARCH FUND

Research fund was provided by the Faculty of Tropical Medicine, Mahidol University, THAILAND.

CHAPTER V

RESULTS

GENERAL INFORMATION

During the study period of June 1995 to April 2003, a total of 170 uncomplicated malarial patients fulfilled the inclusion criteria of this study were admitted to the Hospital for Tropical Diseases, Bangkok or Thongpaphum Hospital, Kanchanaburi province.

Asexual stage of *P. falciparum* was found in 90 cases (52.9%), *P. vivax* in 77 cases (45.3%) and there were only 3 patients (1.8%) with mixed infection.

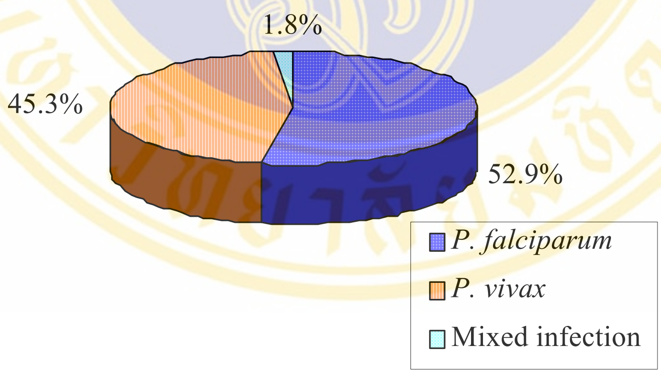

Figure 1. Percentage of malarial species

Table 1. Demographic data of *P. vivax* and *P. falciparum* patients

Demographic data	*P. vivax* (N=77)	*P. falciparum* (N=90)	P value
Mean age (SD) (yr)	6.2 (4.2)	8.7 (3.1)	0.001*
Gender (male: female)	1.6:1	1:1	0.20
Mean weight (SD) (kg)	17.6 (7.7)	23.3 (8.3)	<0.001*
Mean height (SD) (cm)	112 (22.6)	121 (18.6)	0.005*

* P value<0.05

Mean and standard deviation of age, weight and height between *P. vivax* and *P. falciparum* were shown in table 1, which there were significant differences (P<0.01). However, there was no significant difference between gender (P>0.05). The mean length of admission in all of the patients was 4 days (range 1-21 days).

Table 2. Demographic data of *P. vivax* and *P. falciparum* patients (less and more than 7 years old)

Demographic data	*P. vivax* (N=77)	*P. falciparum* (N=90)	P value
Age <7 years	**41**	**24**	
Gender (Male:Female)	0.9:1	0.7:1	0.91
Weight mean (SD) (kg)	11.4 (3.8)	13.2 (2.9)	0.045*
Height mean (SD) (cm)	89.52 (5.0)	95.6 (11.5)	0.12
Age >7 years	**36**	**66**	
Gender (Male:Female)	3.5:1	1.1:1	0.02*
Weight mean (SD) (kg)	24.28 (4.8)	27.37 (6.2)	0.01*
Height mean (SD) (cm)	128.36 (9.5)	131.52 (9.5)	0.13

* P value<0.05

Comparison of gender, weight and height in age less than and more than 7 years between *P. vivax* and *P. falciparum* patients was shown in table 2. There was no significantly difference between height. However, there was significantly more body weight in *P. falciparum* than *P. vivax* patients. And there were more cases of male in vivax malaria aged greater than 7 years.

CLINICAL MANIFESTATIONS, LABORATORY FINDINGS AND PARASITE COUNT

Table 3. Comparison of clinical manifestations between *P. vivax* and *P. falciparum* patients

Clinical manifestations	*P. vivax* (N=77)		*P. falciparum* (N=90)		P value
	Number	%	Number	%	
Weakness	31/43	72.1	65/73	89.0	0.02*
Chills	47/55	85.4	48/80	60.0	<0.001*
Headache	38/50	76.0	57/76	75.0	0.90
Myalgia	10/18	55.5	6/13	46.1	0.61
Abdominal pain	12/44	27.2	13/67	19.4	0.33
Diarrhea	7/77	9.1	2/85	14.1	0.87
Nausea	29/63	46.0	29/80	36.2	0.24
Vomiting	27/71	38.0	27/84	45.2	0.44
Anorexia	28/77	36.3	39/84	46.4	0.20
Cough	14/37	37.8	12/26	46.1	0.51
Dehydration	8/77	10.3	7/20	35.0	0.01*

* P value<0.05

The clinical manifestations were compared between vivax and falciparum infection by using chi-square test or Fisher's exact test (table 3). It showed that most of the symptoms including headache, myalgia, abdominal pain, diarrhea, nausea, vomiting, anorexia, and cough, were not significant difference between 2 groups (P>0.05). However, weakness and dehydration were significantly more common in *P. falciparum* than *P. vivax* patients. On the other hand, chills was significantly more common in *P. vivax* patients.

Table 4. Vital signs on admission day between *P. vivax* and *P. falciparum* patients

Vital signs	*P. vivax* (N=77)		*P. falciparum* (N=90)		P value
	Mean	SD	Mean	SD	
Maximum temperature (^{o}C)	37.21	0.653	38.55	1.089	<0.001*
Maximum pulse rate (/min)	104.10	4.06	104.39	13.95	<0.001*
Maximum respiratory rate (/min)	24.09	13.69	28.64	6.80	0.95
Systolic blood pressure (mmHg)	100.00	8.09	99.49	9.32	0.74
Diastolic blood pressure (mmHg)	63.02	6.68	61.77	9.44	0.38

* P value<0.05

The mean and standard deviation of vital signs on admission day between *P. falciparum* and *P. vivax* patients were shown in table 4. There were significantly higher temperature and pulse rate in *P. falciparum* than *P. vivax* patients (P<0.01). There were no significant differences between groups, in terms of respiratory rate, systolic blood pressure and diastolic blood pressure.

Table 5. Percentage of hepatomegaly and splenomegaly in *P. vivax* and *P. falciparum* patients

	P. vivax		*P. falciparum*		P value
	Number	%	Number	%	
Hepatomegaly	30/76	39.47	47/90	52.22	0.14
Splenomegaly	25/76	32.89	49/90	54.44	0.02*
Hepatosplenomegaly	23/76	30.26	33/90	36.66	0.48

* P value<0.05

The percentage of hepatomegaly, splenomegaly and hepatosplenomegaly was shown in table 5. There was no significant difference between hepatomegaly in *P. falciparum* and *P. vivax* infection. Hepatosplenomegaly was also not significant difference between *P. falciparum* and *P. vivax* groups. However, splenomegaly was significantly more common in children with *P. falciparum* than *P. vivax* infection.

Table 6. Laboratory findings between *P. vivax* and *P. falciparum* patients

Laboratory	*P. vivax*		*P. falciparum*		P value
	Number	Means (SD)	Number	Means (SD)	
WBC count (x10^9/L)	72	8.7 (4.0)	90	6.8 (2.54)	<0.001*
RBC count (x10^{12}/L)	55	4.07 (0.96)	82	4.3 (0.99)	0.13
Hb (g/dL)	71	13 (18.7)	67	10 (2.03)	0.22
Hematocrit (%)	71	29.07 (6.2)	90	27.18 (6.22)	0.06
Platelet (x10^9/L)	71	158 (82)	89	153 (98)	0.73
Neutrophil (/µL)	67	4680 (2980)	90	4267 (2122)	0.31
Eosinophil (/µL)	71	183 (193)	90	113 (157)	0.01*
Basophil (/µL)	68	44 (83)	90	19 (34)	0.01*
Lymphocyte (/µL)	66	3274 (2289)	90	2057 (981)	<0.001*
Atypical lymphocyte (/µL)	66	88 (136)	90	15 (66)	<0.001*
Monocyte (/µL)	72	532 (418)	90	354 (317)	0.002*
Sodium (mEq/L)	26	135 (5.6)	73	134 (5.39)	0.04*
Potassium (mEq/L)	26	3.7 (0.56)	72	3.8 (0.68)	0.30
Chloride(mEq/L)	26	100 (15.71)	16	103 (2.89)	0.49
HCO_3 (mEq/L)	15	20.8 (4.03)	16	22 (2.92)	0.30
Blood sugar (mg/dL)	23	120.34 (58.50)	84	101.37 (37.23)	0.06
Direct bilirubin (mg/dL)	25	0.22 (0.11)	17	0.29 (0.14)	0.08
Total bilirubin (mg/dL)	24	0.92 (0.57)	84	1.15 (0.65)	0.14
Alk phosphatase (U/L)	25	117.94 (74.47)	76	213.81(183.78)	0.01*
AST (U/L)	25	39.20 (27.06)	87	37.21 (26.28)	0.74
ALT (U/L)	25	23 (17.6)	87	24 (26.69)	0.97

* P value<0.05

Laboratory investigations on admission day were shown in table 6. T-test was used to compare the difference between *P. falciparum* and *P. vivax* groups. The results showed that white blood cell count was significantly lower in *P. falciparum* than *P.vivax* infection (P<0.01). Eosinophil count [mean (SD), 183 (193)] and basophil count [44 (83)] in *P. vivax* group were significantly higher than *P. falciparum* group [mean (SD) 113 (157), 19 (34) respectively]. There were also significant difference of lymphocyte, atypical lymphocyte and monocyte, which were higher in the *P. vivax* group. Sodium level was also significantly lower in the *P. falciparum* group. But alkaline phosphatase was significant higher in the *P. falciparum* group. Red blood cell volume, hematocrit, hemoglobin and platelet count were compared but there were no significant difference between the two groups. Moreover there were also no significant difference in potassium, bicarbonate, blood sugar, total bilirubin, direct bilirubin, AST and ALT level between *P. falciparum* and *P. vivax* infection.

Table 7. Number of patients with abnormal laboratory findings

Laboratory	*P. vivax*		*P. falciparum*		P value
	Number	%	Number	%	
WBC < 4 x 10^9/L	3/72	4.2	6/90	6.7	0.73
WBC > 10 x 10^9/L	23/72	31.9	11/90	12.2	0.004*
Eosinophil > 400/µL	11/71	15.5	7/90	7.8	0.19
Sodium <130 mEq/L	0/26	0.0	15/73	20.5	0.01*
Potassium <3.5 mEq/L	7/26	27.0	17/72	23.6	0.73
Chloride <100 mEq/L	5/26	19.2	1/16	6.2	0.24
HCO_3 <22 mEq/L	8/15	53.3	6/16	37.5	0.37
Blood sugar <75 mg/dL	2/23	8.6	13/84	15.5	0.40
Direct bilirubin >0.2 mg/dL	9/25	36.0	10/17	59.0	0.14
Total bilirubin >0.5 mg/dL	20/24	83.3	71/84	84.5	0.88
Alk phoshatase >360 U/L	0/25	0.0	13/76	17.0	0.02*
AST >40 U/L	7/25	28.0	24/87	27.5	0.96
ALT >35 U/L	5/25	20.0	11/87	12.6	0.35

* P value<0.05

The percentage of abnormal laboratory findings showed in table 7. There was more cases of leucocytosis (>10 X 10^9/L) in *P. vivax* infection. There were also significantly more cases of hyponatremia (<130 mEq/L) and alkaline phosphatemia (>360 U/L) in *P. falciparum* than *P. vivax* infection. However, there were no significant difference in hypokalemia (<3.5 mEq/L), hypochloremia (<100 mEq/L), acidosis (HCO_3 <22 mEq/L), low blood glucose (<75 mg/dL), hyperbilirubinemia and increasing of liver enzymes between falciparum and vivax infection.

There was only 2 in 63 patients had G-6-PD deficiency in *P. vivax* infection and 7 in 86 patients in *P. falciparum* infection, which no significant difference between the two groups (P=0.30).

Table 8. Parasite count per microliter on admission day

Malarial species	Number	Mean	SD
P. vivax	59	8,775.22	9,438.35
P. falciparum	90	58,668.22	61,989.79

P value <0.001

The mean (standard deviation) of parasite count on admission day of *P. vivax* was 8,775.22 (9,438.35) per microliter. On the other hand, that of *P. falciparum* was 58,668.22 (61,989.79) per microliter, which was significantly higher than *P. vivax*. The mean and standard deviation of parasite count of mixed infection count was 9,210 (8,306.12) per microliter for *P. vivax* and 4,060 (4,717.17) per microliter for *P. falciparum*.

THROMBOCYTOPENIA AND MALARIA SPECIES

Table 9. Platelet count in children with *P. vivax* and *P. falciparum* infection

Thrombocytopenia (/µL)	*P. vivax* (N=71)		*P. falciparum* (N=89)		P value
	Number	%	Number	%	
Platelet count <150,000	39	55.0	48	54.0	0.97
Platelet count <100,000	20	28.2	32	36.0	0.38
Platelet count <50,000	4	5.6	12	13.4	0.17

Platelet count was performed in 71 of 77 with vivax malaria and in 89 of 90 with falciparum malaria on admission day. Thrombocytopenia was not significant difference between both groups. However moderate and severe thrombocytopenia (<100,000/µL and < 50,000/µL) were more common in *P. falciparum* infection.

ANEM IA AND MALARIA SPECIES

Table 10. Hematocrit in children with *P. vivax* and *P. falciparum* infection

Hematocrit	*P. vivax* (N=71)		*P. falciparum* (N=90)		P value
	Number	%	Number	%	
Hematocrit <30%	38	53.52%	61	67.77%	0.09
Hematocrit <20%	5	7.04%	11	12.22%	0.41

There was no significant difference of anemia between *P. vivax* and *P. falciparum* groups. However the percentage of anemia in *P. falciparum* group was slightly greater than *P. vivax* group.

OUTCOME OF *P. VIVAX* INFECTIONS

Table 11. Outcome of chloroquine treated *P. vivax* infection according to time sequence

Year	Number of cases	Fever clearance time (h)		Parasite clearance time (h)	
		Number	Mean (SD)	Number	Mean (SD)
1997	26	22	36.4 (28)	26	56.6 (19.39)
1998	7	6	25.3 (9.15)	7	56.14 (19.48)
1999	16	15	30.8 (26.1)	16	56.39 (27.28)
2000	19	17	48 (27.2)	18	60.63 (28.82)
2001	7	7	36 (29.4)	7	65.71 (15.03)
2002	1	1	40	1	81
2003	1	1	9.3	0	-
Total	77	70	36.7 (26.4)	75	58.6 (23.1)

From 1997 to 2003, 77 patients were infected by *P. vivax*, diagnosis was established by thick and thin smears of peripheral blood films and examined microscopically for presence of parasites. The parasites count ranged from 41 to 41,639 parasites per microliter. All patients received oral chloroquine in total of 25 mg base/kg over 3 days, and 92.2 % of patients received primaquine 0.3 mg base/kg once a day for 14 days.

The outcome of these *P. vivax* infection treated with chloroquine was evaluated in aspect of treatment efficacy. The mean of fever clearance time (FCT) was 36.7 hours and the mean of parasite clearance time (PCT) was 58.6 hours. The cure rate was 100% and neither recrudescence nor relapse was observed during the follow-up period of 28 days.

Table 12. Comparison of chloroquine treatment outcome before 2000 and after 2000

Out come of treatment	Before 2000		After 2000		P value
	Number	Mean (SD)	Number	Means (SD)	
Fever clearance time (h)	43	32 (25.43)	27	42 (27.46)	0.14
Parasite clearance time (h)	49	56 (21.8)	26	62 (25.26)	0.26

When fever clearance time and parasite clearance time were evaluated according to time before and after 2000, there was no significant difference between these 2 periods, however FCT and PCT seemed to be longer after the year 2000.

OUTCOME OF *P. FALCIPARUM* INFECTIONS

Fifty-four patients with *P. falciparum* infection received quinine 10 mg/kg every 8 hours for 4 days and then 15 mg base/kg every 8 hours orally for another 4 days (Q4-4). The other 31 patients were treated with artesunate rectocap 10-19 mg/kg/d once a day for 3 days followed by mefloquine 25 mg base/kg divided into 2 doses.

Table 13. Outcome of *P. falciparum* malaria treated with quinine and artesunate plus mefloquine regimen

Outcome	**Quinine**		**Artesunate + Mefloquine**		**P value**
	Number	**Mean (SD)**	**Number**	**Mean (SD)**	
Fever clearance time (h)	54	68 (34.78)	29	39 (24.88)	<0.001*
Parasite clearance time (h)	48	66 (41.65)	29	50 (18.41)	0.002*

* P value <0.05

The outcome of quinine and artesunate treatment was shown in table 14. The mean (SD) of fever clearance time and parasite clearance time with quinine were 68(34.78) and 66 (41.65), respectively. The cure rate of quinine treatment was 75.4%. Eleven patients (24.6%) failed by this treatment regimen. For 29 patients with artesunate and mefloquine treatment, the mean (SD) of fever and parasite clearance time were 39 (24.88) and 50 (18.41). The cure rate was 91.6%. Only 2 patients (8.33%) had reappearance of asexual *P. falciparum* on the day 20.

When the outcome of *P. falciparum* was compared according to the regimen of treatment, it showed that fever clearance time and parasite clearance time in artesunate and mefloquine group was shorter than quinine group significantly ($P<0.01$ both). However cure rate was not significant difference between these 2 regimens of treatment ($P=0.20$).

CHAPTER VI

DISCUSSION

Malaria is found in over 100 countries in Africa, Asia, and the Americas. However, the majority (about 80%) of malaria cases occur in Africa and this due to infection with the most deadly human malaria parasite *Plasmodium falciparum*. Unfortunately, more than half of fatal cases occurred among African children between 6 months and 5 years of age. One important step in fighting against this infection still remains the prompt diagnosis followed by a sufficient treatment of the disease since both measures are able to reduce the high incidence of malaria-related mortality (Pasvol,1998). This study was performed in order to compare the clinical manifestations, the laboratory findings and to describe outcome of uncomplicated malaria.

Analysis of the clinical manifestations of *P. falciparum* and *P. vivax* malaria patients showed that patients in both groups presented with fever, headache, myalgia, abdominal pain, nausea, anorexia and cough. There was no significant difference between *P. falciparum* and *P.vivax* infection. These finding are in accordance with previous studies revealing similar symptoms between *P. falciparum* and *P. vivax* patients (Brooks et al., 1967). However, in the present study there were significant differences in general weakness and dehydration. These two clinical symptoms were more frequent in the patients with *P. falciparum* infection compared to those being infected with *P. vivax.* Despite intensive clinical malarial research and studies during the last decades, only very limited data was available comparing the clinical manifestations in both, *P. falciparum* and *P. vivax* patients. The few clinical reports described weakness and dehydration in both, *P. falciparum* and *P. vivax* patients, however they leave the putative reasons open why these clinical manifestations differ. A possible explanation might be that there was more pronounced anorexia in *P. falciparum* patients which may cause more weakness and dehydration in falciparum malaria when compared with *P. vivax* cases.

Furthermore, in this study hepatomegaly was found in 52.22% of patients infected with *P. falciparum* in contrast to 39.47% with *P. vivax* infection. Ahmad et al. (1987) showed hepatomegaly and splenomegaly were more prevalent in *P. vivax* patients (P < 0.001 and P < 0.01, respectively). However, Aikawa et al. (1980) reported that the tissue reactions of the liver system was similar in all forms of malaria but tended to be more pronounced in *P. falciparum* infections. Thus, the liver was enlarged as a result of edema in early infection and also a marked sinusoidal dilatation and congestion with Kupffer cells (Sowunmil, 1996).

In acute falciparum malaria, the spleen was enlarged because erythrocyte containing parasite may nipped off during migration through the splenic cords, a process known as pitting and adhere to macrophages via their surface knobs (White, 1992). In this present study, higher percentage of splenomegaly was also found in *P. falciparum* compared to the *P. vivax* group (54% versus 33%). In contrast Ahmad et al. (1987) reported more frequently splenic enlargement in *P. vivax* infections.

In comparing the laboratory finding between *P. falciparum* and *P. vivax,* we showed that white blood cell, monocyte and basophil were significant lower in *P. falciparum* than *P. vivax* infection. Ladhani et al. (2002) described that leucopenia was common in uncomplicated falciparum malaria but not associated with death. Previous studies had included only children with severe malaria and showed that leucocytosis was associated with severe anemia, hypoglycemia and death (Warrell et al., 1982; Modiano et al., 2001). The low monocyte count and basophil count in children with *P. falciparum* may associate with low white blood cell count (Ladhani et al., 2002). Hyponatremia were as well significant prevalence in *P. falciparum* than *P. vivax*. Brooks et al. (1967) and Miller et al. (1967) explained that mild hyponatremia was common in falciparum. Miller et al. (1967) found variable responses to the water load test in hyponatremic patients with normal (blunted) responses were considered to have inappropriate secretion of antidiuretic hormone although urinary sodium concentration were all low. The fall in plasma sodium resulting from changes in membrane transport (Dunn, 1969). Other study explained that hyponatremia was related to anorexia, vomiting and diarrhea in some patients (Aikawa et al., 1980). In this study showed eosinophil count in falciparum malaria was significantly lower than vivax malaria. Sanderson et al. (1985) and Lucey et al.

(1996) showed that low eosinophil count might be a result of the Th-2 immune response and apparently by other immune pathways as well. The balance between Th-1 and Th-2 mediated immune responses is of central importance for the body's response to parasitic infection therefore decreased eosinophil counts on day 0 may be a reflection of an ongoing Th-1 response due to acute infection which suppresses Th-2.

The results revealed that lymphocyte and atypical lymphocyte count were significant lower in *P. falciparum* than *P. vivax*. The marked decrease in the number of circulating lymphocytes, particularly of the T-cell population in acute human malaria has stimulated research to explain this finding (Kern et al., 2000). Peripheral T lymphopenia is a well-established feature of patients with falciparum malaria although its cause remains unclear (Matsumoto et al., 2000). It seemed likely that the cause of T lymphopenia in malaria was not singular but multiple and the major causes differ from each other, since malaria infection induced a wide variety of host reactions in each host-parasite system. We speculated further that the major cause of T lymphopenia changes during the course of infection even in the early phase of disease. T lymphopenia was mainly due to reallocation of activated T cells Fas-mediated apoptosis played an increasingly important role in T lymphopenia (Hirsch et al., 1999).

The other hematologic results of this study were not significant difference between *P. falciparum* and *P. vivax* groups such as red blood cell count, hemoglobin, hematocrit, platelet count, neutrophil count, chloride, bicarbonate, blood sugar, direct bilirubin, total bilirubin, AST and ALT which were in normal range because of inclusion and exculsion criteria of study. However, anemia (hematocrit <30%) and moderate anemia (hematocrit <20%) were more common in *P. falciparum* malaria. Another study also showed similar findings, anemia was seen more in *P. falciparum* (65.1%) compared to *P. vivax* (44.6%) cases (Layla et al., 2002). The pathogenesis of anemia in malaria was extremely complex, multifactorial and incompletely understood. It was thought to result from a combination of hemolysis of parasitized red blood cell, accelerated removal of both parasitized and innocently unparasitized red blood cell, depressed as well as ineffective erythropoiesis with dyserythropoietic changes, and anemia of chronic disease (Woodruff et al., 1979). Other factors contributing to anemia in malaria included decrease red blood cell deformability, splenic phagocytosis and/or pooling thus they have an increased rate of clearance from the circulation (Conrad, 1969). About thrombocytopenia, there were many previous studies explain the mechanism of thrombocytopenia in malaria, which supported the more prevalence and more severe of thrombocytopenia were found in *P. falciparum* infection. Our study also supported that severe and moderate thrombocytopenia were more prevalence in *P. falciparum* infection, but there was no significant difference between *P. vivax* and *P. falciparum* infection. It might be possible to show a significant difference by increase sample size of the study. Similar to previous study they found more percentage of thrombocytopenia in *P. falciparum* than *P. vivax* groups (Moulin et al., 2003; Echeverri et al., 2003). The mechanisms of thrombocytopenia in malaria was both non-immunological destruction as well as immune mechanisms involving specific platelet-associated immunoglobulin G antibodies that bind directly to the malarial antigen in the platelets to play a role in the lysis of platelets and the development of thrombocytopenia (Yamaguchi et al., 1997). Furthermore estimates of platelet-associated immunoglobulin G may be raised by platelet fragmentation which exposes non-specific intracellular binding site (Schulman et al., 1982). It is possible therefore that in vivo platelet activation and aggregation occur in acute malaria with accelerated splenic removal of aggregated or degranulated platelets (Looareesuwan et al., 1992). For G-6-PD analysis, we found seven patients with G-6-PD deficiency in *P. falciparum* infection and only two patients in *P. vivax* infection. The mechanism of relative resistance against malaria in G-6-PD deficiency was during intraerythrocytic development, the ring form parasite needs to use host G-6-PD for development into a trophozoite and subsequently into a schizont development (Marina et al., 1998).

From 1997 to 2003, 77 cases of *P. vivax* infection were evaluated the outcome of treatment in aspect of fever clearance time, parasite clearance time and cure rate. Though all cases were cured and there were no significant differences of fever clearance time and parasite clearance time between the period before and after 2000. Another study also showed similar findings chloroquine was sensitive with *Plasmodium vivax* in Thailand (Looareesuwan et al., 1999). However fever clearance time and parasite clearance time of years after 2000 seem to be longer than before

2000. This might be the warning sign of the developing of chloroquine resistance *P. vivax* in Thailand. A previous report showed three (11%) of 27 *P. vivax* patients in Colombia failed in treatment with the standard chloroquine regimen (Soto et al., 2001). The inability to eliminate parasitemia by chloroquine could be due to recrudescence of *P. vivax* blood stages, or relapse from *P. vivax* liver stages (Baird et al., 1996). In addition, Pukrittayakamee et al.(1994) reported that chloroquine resistance may be masked because it is often given in combination with primaquine, which also has some effect on asexual blood stages of *P. vivax*.

In this study, the outcome of 54 patients received oral quinine and 29 patients received artesunate and mefloquine was revealed that fever and parasite clearance time were shorter than quinine group. Similar to the previous reported also found both fever and parasite clearance time were shorter in artesunate group (Huda et al., 2003). It is because artesunate is highly and rapidly effective against malaria parasites including multi-resistant strains of *P. falciparum* (Guan et al., 1982). However this study could not show the effectiveness in term of cure rate, this might be because of low number of patients.

CHAPTER VII

CONCLUSION

This study was the retrospective study about clinical manifestations, laboratory findings and also outcome of uncomplicated falciparum malaria and vivax malaria in Thai children from 1991-2003. We were able to show that 52.9% of cases with *P. falciparum*, 45.3% with *P. vivax* and 1.8% (3 patients) with mixed infection. The clinical manifestations were revealed that weakness and dehydration were more common in *P. falciparum* than *P. vivax* infection. Moreover, we also found out that splenomegaly was more common in falciparum malaria. In comparing the laboratory finding showed that white blood cell, eosinophil, basophil, lymphocyte, atypical lymphocyte, monocyte and sodium was significant lower in *P. falciparum* than *P. vivax* infection. There was also significant higher alkaline phosphatase in *P. falciparum* than *P. vivax* infection. Thrombocytopenia and anemia were no significant difference between these 2 groups. However, the percentage of thrombocytopenia and anemia in children with *P. falciparum* were higher than *P. vivax* infection. The outcome of treatment in 77 cases of *P. vivax* infection, all cases were cure and there were not significant differences of fever clearance time and parasite clearance time according to time sequence but they seems to be longer in the recent years. For the outcome of falciparum malaria, fever clearance time and parasite clearance time were shorter in artesunate than quinine group.

BIBLIOGRAPHY

Adelusi. SA, Dawodu AH, Salako LA. Kinetics of the uptake and elimination of chloroquine in children with malaria. Br J Clin Pharmac 1982;14:483-7.

Ahmad SH, Kidwai T, Moonis R, Shahad T, Chandra J. Clinical profile of malaria in children - A prospective study from Aligarh. Ann Trop Paediatr 1987;7:82-6.

Aikawa M, Suzuki M, Gutierrez Y. Pathology of malaria. Am J Trop Med Hyg 1980;42:30-7.

Baird JK, Nalim MFS, Basri H, et al. Survey of resistance to chloroquine by *Plasmodium vivax* in Indonesia. Trans R Soc Trop Med Hyg 1996;90:409-11.

Bloland PB, Lackritz EM, Kazembe PN, et al. Beyond chloroquine: implication of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. J Infect Dis 1993;167:932-14.

Bradley D, Newbold CI, Warrell DA. Malaria. In: Weatherall DJ, Ledingham JGG, Warrell DA, eds. Oxford Text Book of Medicine. 3rd ed. Oxford: University Press, 1996;1:835-63.

Brooks MH, Malloy JP, Bartelloni PJ et al. Pathophysiology of acute falciparum malaria: correlation of clinical and biochemical abnormalities. Am J Med 1967; 43:735-50.

Bunnag D, Karbwang J, Viravan C, Chittamas S, Harinasuta T. Clinical trials of mefloquine with tetracycline. Southeast Asian J Trop Med Public Health 1992;3:337-82.

Clark IA, Al Yaman FM, Jacobson LS. The biological basis of malarial disease. Int J Parasitol 1997;27:1237-49.

Connor DH, Neafie RC, Hockmeyer WT. Malaria. In: Binford CH, Connor DH, eds. Pathology of tropical and extraordinary disease. Washington, D.C: Armed Forces Institute of Pathology, 1976;1:273-83.

Conrad M. Pathophysiology of malaria hematologic observations in human and animal studies. Ann Int Med 1969;70:134-41.

Dunn MJ. Alterations of red blood cell sodium transport during malarial infection. J Clin Invest 1969;48:674-84.

Dwivedi AK, Saxena D, Singh S. HPLC and HPTLC assays for the antimalarial agents Chloroquine, Primaquine and Bulaquine. J Pharm Biomed Anal 2003;33:851-8.

Echeverri M, Tobon A, Alvarez G, Carmona J, Blair S. Clinical and laboratory findings of *Plasmodium vivax* malaria in Colombia, 2001. Rev Inst Med Trop Sao Paulo. 2003;45:29-34.

English MC, Waruiru C, Lightowler C, et al. Hyponatremia and dehydration in severe malaria. Arch Dis Child 1996;74:201-5.

Guan WB, Huang WJ, Zhou YC, Gong JZ. Effect in vitro of artesunate and its derivatives on *Plasmodium falciparum*. Acta Pharmacologica Sinica 1982;3:139-41.

Hall A P The treatment of malaria. BMJ 1976;1:323-8.

Hien TT, Phu NH, Mai NTH, et al. An open randomized comparison of intravenous and intramuscular artesunate in severe falciparum malaria. Trans R Soc Trop Med Hyg 1992;86:584-5.

Hill DR. Health advice for international travel. Ann Intern Med 1988;108:839-52.

Hirsch CS, Toossi Z, Vanham G, et al. Apoptosis and T cell hyporesponsiveness in pulmonary tuberculosis. J Infect Dis 1999;179:945-53.

Huda SN, Shahab T, Ali SM, Afzal K, Khan HM. A comparative clinical trial of artesunate and quinine in children with severe malaria. Indian J Pediatr 2003;40:939-45.

Karbwang J, Na Bangchang K, Thanavibul A, Bunnag D, Chongsuphajaisiddhi T, Harinasuta T. Comparison of oral artemether and mefloquine in acute uncomplicated falciparum malaria. Lancet 1992;340:1245-8.

Kern P, Dietrich M, Hemmer C, Wellinghausen N. Increased levels of soluble Fas ligand in serum in *Plasmodium falciparum* malaria. Infect Immun 2000;68:3061-3.

Ladhani S, Lowe B, Cole AO, Kowuondo K, Newton CR. Changes in white blood cells and platelets in children with falciparum malaria: relationship to disease outcome. Br J Haematol 2002;119:839-47.

Layla AM, Bashawri FC, Ahmed A, et al. Hematological aspects. Ann Trop Med 2002;22:5-6.

Looareesuwan S, Davis JG, Allen DL, Lee SH, Bunnag D, White NJ. Thrombocytopenia in malaria. Southeast Asian J Trop Med Public Health 1992;23:1-7.

Looareesuwan S, Wilairatana P, Vanijanonta S, Viravan C. Efficacy and tolerability of sequential, artesunate suppository plus mefloquine, treatment of severe falciparum malaria. Ann Trop Med Parasitol 1995;89:469-75.

Looareesuwan S, Wilairatana P, Krudsood S et al. Chlororoquine sensitivity of *Plasmodium vivax* in Thailand. Ann Trop Med Parasitol 1999;93:225-30.

Lucey DR, Clerici M, Shearer GM: Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Micro Rev 1996;9:532-62.

Marina C, Giuliana G, Estella O, et al. Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD) deficient erythrocytes parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. Blood 1998;92:2527-34.

Maegraith BG. Interdependence: The pathological of malaria. Am J Trop Med Hyg 1977;26:344-54.

Matsumoto J, Kawai S, Terao M, et al. Malaria infection induces rapid elevation of the soluble Fas ligand level in serum and subsequent T lymphopenia. Infect Immun 2000;68:1183-8.

Mehta KS, Halankar AR, Makwana PD, Jorane PP, Satija PS, Shah VB. Severe acute renal failure in malaria. J Postgrad Med 2001;47:24-6.

Migasena S. Hypoglycaemia in falciparum malaria. Ann Trop Med Parasitol 1983;77:323-4.

Miller LH, Makaranond P, Sitprija V, Suebsanguan C, Canfield CJ. Hyponatremia in malaria. Ann Trop Parasitol 1967;61:265-79.

Modiano D, Sirima BS, Konate A, Sanou I, Sawadogo A. Leucocytosis in severe malaria. Trans R Soc Trop Med Hyg 2001;95:175-6.

Moulin F, Lesage F, Legros AH, et al. Thrombocytopenia and *Plasmodium falciparum* malaria in children with different exposures. Arch Dis Child 2003;88:540-1.

Murphy GS, Basri H, Purnomo, et al. Vivax malaria resistant to treatment and prophylaxis with chloroquine. Lancet 1993;34:96-100.

Oh MD, Shin H, Shin D. Clinical features of vivax malaria. Am J Trop Med Hyg 2001;65:143-6.

Pasvol G. The treatment of falciparum malaria in African children. Afr Health 1998;20:19-20.

Phillips RE, Warrell DA, White NJ, et al. Intravenous quinine for the treatment of severe falciparum malaria.Clinical and pharmacokinetic studies. N Eng J Med 1985;312:1273-8.

Pukrittayakamee S, Vanijanonta S, Chantra A, Clemens R, White NJ. Blood stage antimalarial efficacy of primaquine in *Plasmodium vivax* malaria. J Infect Dis 1994;169:932-5.

Sabchareon A, Chongsuphajaisiddhi T, Singhasivanon V, Chanthavanich P, Attanath P. In vivo and vitro responses to *Plasmodium falciparum* infection in children to quinine and quinidine. Bull WHO 1988;66:347-52.

Sanderson CJ, Warren DJ, Strath M. Identification of a lymphokine that stimulates eosinophil differentiation in vitro. J Exp Med 1885;162:60-74.

Schulman NR, Leissinger CA, Hotchkiss A, Kautz C. An in-vivo model demonstrating that elevated platelet-associated IgG is a non-specific consequence of platelet destruction. Blood 1982;60:191-8.

Schwartz E, Regev-Yochay G, Kurnik D. A consideration of primaquine dose adjustment for radical cure of *Plasmodium vivax* malaria. Am J Trop Med Hyg 2000;62:393-5.

Soto J, Toledo P, Gutierrez M, et al. *Plasmodium vivax* clinically resistant to chloroquine in Columbia. Am J Trop Med Hyg 2001;65:90-3.

Sowunmil A. Hepatomegaly in acute falciparum malaria in children. Trans R Soc Trop Med Hyg 1996;90:540-2.

Warrell DA, Looareesuwan S, Warrell MJ, et al. Dexamethasone proves deleterious in cerebral malaria: a double-blind trial in 100 comatose patients. N Eng J Med 1982;306:313-9.

Warrell DA, Molyneux ME, Beales PF. Severe and complicated malaria. Trans R Soc Trop Med Hyg 1990;84:1-65.

Warrell DA. Cerebral malaria. Schweiz Med Wochenschr 1992;122:879-6.

White N J, Warrell DA, Chanthavanich P, et al. Severe hypoglycemia and hyperinsulinemia in falciparum malaria. N Eng J Med 1983;309:61-6.

White N J. Clinical pharmacokinetics of antimalarial drugs. Clinl Pharmacokinet 1985;10:187-215.

White N J. Malaria: pathophysiology. Clin Trop Med and Com Dis 1986;1:55-89.

White N J. Antimalarial drug resistance: the pace quickens. J Antimicrob Chemother 1992;30:571-85.

White N J, Ho M. The pathophysiology of malaria. Adv Parasitol 1992;31:83-173.

WHO. Development of recommendations for the protection of short-stay traveler to malaria endemic areas: Memorandum from two WHO meetings. Bull WHO 1988;66:177-96.

WHO. World malaria situation in 1990 part II. Wkly Epidemiol Rec 1992;22:161-7.

WHO. Severe and complicated malaria. Trans R Soc Trop Med Hyg 2000;94:1-15.

Woodruff A, Amsdell V, Pettitt L. Cause of anemia in malaria. Lancet 1979;1:1055-7.

Yamaguchi S, Kubota T, Yamagishi T, et al. Severe thrombocytopenia suggesting immunological mechanism in two cases of vivax malaria. Am J Hematol 1997;56:183-6.

APPENDIX A

CRITERIA FOR SEVERE AND COMPLICATED MALARIA

(WHO, 2000)

CRITERIA FOR SEVERE AND COMPLICATED MALARIA

(WHO, 2000)

a. Cerebral malaria

b. Severe anemia (hematocrit < 15%)

c. Renal failure

d. Pulmonary edema (radiological)

e. Hypoglycemia (blood sugar < 2.2mmol/L or < 40mg/dL)

f. Circulatory collapse, shock

g. Spontaneous bleeding and disseminated intravascular coagulation (DIC)

h. Repeated generalized convulsions

i. Acidosis (arterial pH <7.25 or plasma bicarbonate < 15mmol/L)

j. Macroscopic hemoglobinuria

k. Hyperparasitemia

l. Hepatic dysfunction (jaundice)

A patient was classified as severe malaria if schizontemia was detected in peripheral blood.

APPENDIX B

OPERATIONAL DEFINITIONS

OPERATIONAL DEFINITIONS

Fever: Body temperature $\geq$ 37. 5^{o} C (oral)

Anemia: Hemoglobin < 10 g/dL or hematocrit < 30.0%

Mild anemia: Hematocrit 20.0% - 30.0%

Moderate anemia: Hematocrit 15.0% - 19.9%

Severe anemia: Hematocrit <15.0%

Fever clearance time (FCT)**:** The time from beginning of antimalarial treatment until the body temperature falls to normal (<37.5^{o} C) and remained at that value for at least 48 hours.

Parasite clearance time (PCT)**:** The time from beginning of antimalarial treatment and the first negative blood slide which remained negative for at least 48 hours.

Cure: Negative peripheral blood smear for asexual parasite at 28-day of follow-up.

Antibiotics: Patients receiving any of the following antibiotics within 3 days prior to admission were excluded: sulphonamides, bactrim, tetracycline, doxycycline, macrolides, rifampicin, chloramphenicol and fluoroquinolones.

APPENDIX C

DATA RECORD FORM

DATA RECORD FORM-A

1. Admission number [ ][ ][ ][ ]/ [ ][ ]
2. Date of admission [ ][ ]/[ ][ ]/[ ][ ] (D/M/Y)
3. Date of discharge [ ][ ]/[ ][ ]/[ ][ ] (D/M/Y)
4. Age [ ][ ]/[ ][ ] (Y/M)
5. Sex (male = 1, female = 2) [ ]
6. Height (cm) [ ][ ][ ].[ ][ ]
7. Body weight (kg) [ ][ ][ ].[ ][ ]
8. Date of illness (onset of fever) [ ][ ]/[ ][ ]/[ ][ ] (D/M/Y)
9. Previous malaria infection [ ] No [ ] Yes If yes, last infection ____________
10. Species of malaria infection [ ] Pv [ ] Pf [ ] Mixed
11. Antimalarial regimen
    - [ ] Quinine + Tetracycline
    - [ ] Quinine (Q4-4)
    - [ ] Artesunate + Mefloquine
    - [ ] Halofantrine
    - [ ] Artemether + Lumefantrine
    - [ ] Malarone®
    - [ ] Chloroquine
    - [ ] Chloroquine + Primaquine
    - [ ] Others________________________
12. Onset of treatment (D/M/Y) [ ][ ]/[ ][ ]/[ ][ ] hr:min [ ][ ]:[ ][ ]
13. Fever clearance Time [ ][ ].[ ][ ] hr [ ] Missing
14. Parasite clearance time Pf __________ hr. Pv ___________hr. [ ] Missing
15. Cure [ ] Yes [ ] No [ ] Missing

DATA RECORD FORM-B

Symptoms and signs	D0	D1	D2	D3	D4	D5	D6	D7
Max. body temperature (° C)								
Respiratory rate (/minute) (maximum)								
Pulse rate (/minute) (maximum)								
Systolic blood pressure (mm Hg) (minimum)								
Diastolic blood pressure (mm Hg) (minimum)								
Weakness (N=0, Y= 1)								
Chills/Rigor (N=0, Y= 1)								
Headache (N=0, Y= 1)								
Myalgia (N=0, Y= 1)								
Abdominal pain (N=0, Y= 1)								
Diarrhea (N=0, Y= 1)								
Nausea (N=0, Y= 1)								
Vomiting (N=0, Y= 1)								
Anorexia (N=0, Y= 1)								
Cough (N=0, Y= 1)								
Hydration (No dehydration=0, Dehydration = 1)								
Itching (N=0, Y= 1)								

Liver (cm) [ ][ ].[ ][ ]

Spleen (cm) [ ][ ].[ ][ ]

DATA RECORD FORM-C

Laboratory finding	**Do**	**D 7±1**	**D 28±2**
WBC count (x10^9/L)			
RBC count (×10^{12}/L)			
Hemoglobin (g/dL)			
Hematocrit (%)			
Platelet count (/µL)			
Band (%)			
Neutrophil (%)			
Eosinophil (%)			
Basophil (%)			
Lymphocyte (%)			
Atypical lymphocyte (%)			
Monocyte (%)			
Sodium (mEq/L)			
Potassium (mEq/L)			
Chloride (mEq/L)			
Serum HCO_3 (mEq/l)			
Blood sugar (mg/dL)			
Direct bilirubin (mg/dL)			
Total bilirubin (mg/dL)			
Alk phosphatase (U/L)			
AST(U/L)			
ALT(U/L)			

G-6-PD status [ ] Normal [ ] Deficiency [ ] No data

DATA RECORD FORM-D (Parasite count)

Actual date (day/month/year)	Actual time 24 hr (hour:minute)	*P. falciparum*			*P. vivax*		
		/1000RBC	/200WBC	/µL	/1000RBC	/200WBC	/µL

BIOGRAPHY

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SEX: FEMALE

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