EFFECT OF PRIMAQUINE STANDARD DOSE (15 MG/DAY FOR 14 DAYS) IN THE TREATMENT OF VIVAX MALARIA PATIENTS IN THAILAND

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Abstract. Primaquine (8-aminoquinoline), the only effective drug to prevent relapses of the persistent liver forms of *Plasmodium vivax* and *Plasmodium ovale*, can induce hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. The severity varies considerably among affected individuals. Three hundred and sixty-four *Plasmodium vivax* cases (342 G6PD-normal and 22 G6PD-deficient) were given a 3-day course of chloroquine (total dose 1,500 mg) followed by primaquine 15 mg a day for 14 days and completed a 28-day follow-up.

All G6PD-deficient patients were male; there were no relapses or serious adverse events during the study. Although a significant decrease in hematocrit levels and an increase in the percent reduction of hematocrit levels were observed on day 7 (34.9±5.0 *vs* 26.7±5.4; (-1.2)±14.4 *vs* (-24.5) ±13.9 respectively) and on day 14 (35.7±4.3 *vs* 30.9±3.1; 1.6±17.8 *vs* (-11.0) ±19.3 respectively) blood transfusion was not required. Daily doses of 15 mg of primaquine for 14 days following a full course of chloroquine when prescribed to Thai G6PD deficient patients where *Mahidol* variant is predominant, are relatively safe.

INTRODUCTION

Plasmodium vivax causes a clinically benign illness but relapse is frequent; it is a common cause of malaria in Asia and South America. Currently, the treatment of choice is a 3-day course of chloroquine in a total dose of 1,500 mg followed by primaquine, 15 mg a day for 14 days. Primaquine is the only effective drug to prevent relapses of the persistent liver forms of *Plasmodium vivax* or *Plasmodium ovale*, and can be used to block *Plasmodium falciparum* transmission by killing gametocytes. However, primaquine - which has been used clinically for almost 50 years (Peters, 1980; Karbwang and Harinasuta, 1992) - may cause severe hemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency whose geographical distribution is correlated with highly endemic areas of malaria (WHO, 1997). This condition is very important in clinical practice, especially in malaria patients, because deficient G6PD enzyme activity may lead to altered host responses to environmental factors including infections and drugs. Infection- or drug-induced hemolysis may be very severe leading to hemoglobinuria, acute renal failure and death (Na-Nakorn *et al*, 1970). The severity of hemolysis is directly related to the degree of G6DP deficiency and to the quantity of primaquine administered. The degree of G6PD-deficient varies greatly, ranging from mild in Africans (African variant A^-) to very severe in

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people of Eastern Mediterranean and West Asian descent (Mediterranean variant B$^-$) (Clyde, 1981). There are at least eight G6PD variants in Thailand; G6PD *Mahidol* is the most common (Panich *et al*, 1972). Since most of the *vivax* malaria-infected patients are treated as out-patients, primaquine might be prescribed simultaneously with chloroquine on the day of consultation without testing for G6PD activity, especially in remote areas.

We compared clinical findings in G6PD-deficient male patients with G6PD-normal patients in order to analyse the pattern of acute hemolysis in G6PD-deficient in those treated with a standard regimen (chloroquine followed by primaquine) for *Plasmodium vivax* infection.

MATERIALS AND METHODS

This study was carried out at the Hospital for Tropical Diseases, Bangkok, Thailand. *Vivax* malaria patients admitted to the hospital between April 1992 and December 1997 were enrolled if they were 12-60 years old, weighed over 30 kg, signed a consent form, and agreed to remain in the hospital for 28 days to exclude reinfection. Reasons for exclusion included pregnancy, history of antimalarial drug treatment within the preceding 2 weeks, mixed *Plasmodium vivax* and *Plasmodium falciparum* infections or unwillingness to remain hospitalized for at least 28 days. Approved was given by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

All patients received a standard regimen of 1,500 mg chloroquine phosphate (from the Government Pharmaceutical Organization of Thailand, in tablets containing 150 mg base each) over 3 days: 600 mg base initially, followed by 300 mg base at 6, 24, and 48 hours, giving a total dose of approximately 20 mg/kg; this was followed by primaquine 15 mg daily for 14 days.

Oral temperature, pulse, and respiratory rates were measured every 4 hours and blood pressure was measured daily. Monitoring of signs and symptoms of malaria was performed daily for the first 7 days of admission and weekly thereafter. All patients were closely monitored for the clinical signs of intravascular hemolysis and hemoglobinuria. Fever clearance time was defined as the time from the start of treatment until the oral temperature dropped below 37.5°C for at least 48 hours.

Laboratory tests included a complete blood count and blood chemistry (liver function tests, blood urea nitrogen, creatinine) and urinalysis were performed before treatment and repeated weekly until discharge. The degree of glucose-6-phosphate dehydrogenase activity was estimated by the fluorescent spot test (Beutler). Whole blood is added to a mixture of glucose-6-phosphate (G6P), NADP, saponin, and buffer, and a spot of this mixture is placed on filter paper and observed for fluorescence with ultraviolet light. If G6PD is present, NADP is converted to NADPH. Since phosphogluconate is present in most hemolysates, NADPH fluoresces but NADP does not. The normal control sample fluoresces brightly - lack of fluorescence indicates G6PD - deficient. By reoxidizing any small amounts of NADPH formed, oxidized glutathione (GSSG) enhances the ability of the test to detect mild G6PD deficient. This is the recommended screening test for G6PD deficient (Beutler *et al*, 1979).

Thick and thin films were obtained from fingerpricks and stained with Giemsa. Blood smears were examined every 12 hours from initiation of treatment until they were negative on two consecutive occasions; thereafter blood smears were done daily until discharge. Blood films were deemed negative if no parasites were seen in 200 oil-immersion fields on a thick blood film. Parasitemias (asexual parasite/μl blood) were determined by counting the number per 200 white blood cells (thick film) or the number per 1,000 red blood cells (thin film). Parasite clearance time was expressed in hours from the start of treatment until the parasite counts fell below the level of detection for at least 24 hours.

Chloroquine 'responsiveness' was defined as the absence of *Plasmodium vivax* parasitemia during the 28-day follow-up period, whereas chloroquine 'failure' meant either failure to clear initial *vivax* parasitemia within 7 days or the reappearance of *vivax* parasitemia within 28 days of starting therapy. Patients in whom *Plasmodium vivax* parasitemias re-appeared during the follow-up period were retreated with the same regimen and monitored for an additional 28 days. If a patient developed severe hemolytic anemia, blood transfusion and the hospital's standard supportive treatment were given.

Statistical analysis

Descriptive statistics and statistical analysis were conducted using the Epi Info Version 6.04 (USD Inc, Stone Mountain, GA) software package. Comparisons were made using χ^2 test and Student's *t*-test. A p-value <0.05 was considered statistically significant.

RESULTS

Five hundred and ninety-three patients with acute *vivax* malaria were enrolled in the study, 34 were G6PD deficient (30 males and 4 females). The demographic, clinical and pretreatment characteristic data were comparable (except gender) (Table 1). The majority of the patients had contracted the infection at the Thai-Myanmar border primarily in Kanchanaburi, Ratchaburi, and Tak provinces (63% and 62% in G6PD-normal and G6PD-deficiency groups respectively). After treatment, 229 (37%) patients [217 (39%) G6PD-normal and 12 (35%) G6PD-deficient], withdrew from the study for social reasons unrelated to drug treatment or side-effects. However, before leaving the hospital, they were well with no parasitemia. A total of 364 patients completed a 28-day follow-up. Only the patients who were followed for 28 days were included in the analysis.

Of 364 patients, 342 had normal and 22 had deficient G6PD activity. All patients in the G6PD-deficient group were males. There were no serious adverse events from primaquine (including severe hemolysis, cyanosis, abdominal cramp, hypertension, arrhythmia, central nervous system symptoms, granulocytopenia, agranulocytosis and leukocytosis) in the G6PD-deficient group. However, during the follow-up period, the hematocrit levels of the G6PD-deficient group were significantly lower on day 7 and day 14, returning to the baseline level on day 21 and finally becaming higher on day 28 when compared with G6PD-normal patients (Fig 1). The percent reduction of hematocrit level had a similar pattern (Fig 2). The minimum hematocrit level was 20% in the G6PD-deficient group; it was 14% in the G6PD-normal group. No patient in the G6PD-deficient group required blood transfusion or other medical intervention. On the other hand, blood transfusions were required for three patients in the G6PD-normal group.

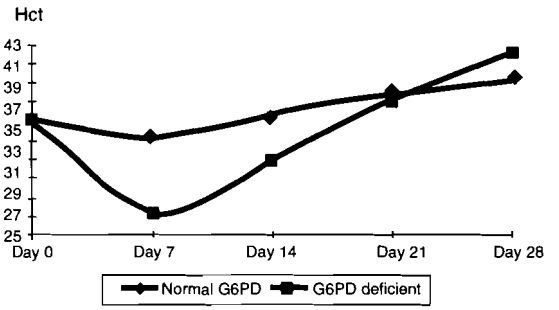

Fig 1–Hematocrit levels of the G6PD-normal and G6PD-deficient groups.

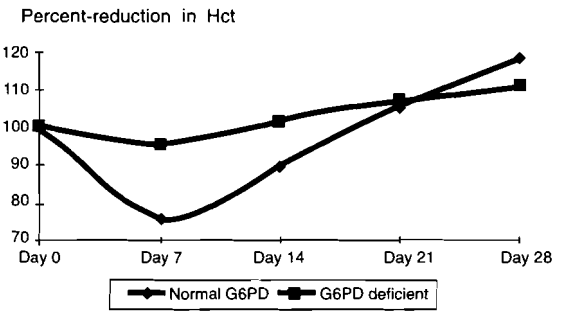

Fig 2–Percent reduction in hematocrit of the G6PD-normal and G6PD-deficient groups.

Table 1
Baseline characteristics of G6PD-normal and G6PD-deficient patients.

	G6PD-normal (n=559)	G6PD-deficient (n=34)	p-value
Age (years)			
Means±SD	25.1±9.1	23.0±7.9	0.19
Min-max	(12-63)	(15-48)	
Sex (Male:Female)	343:216	30:4	<0.01
Height (cm)			
Means±SD	159.1±8.1	60.8±7.5	0.22
Min-max	(138-187)	(144-173)	
Weight (kg)			
Means±SD	51.7±8.7	51.5±9.2	0.88
Min-max	(30-88)	(30-72.5)	
First malarial attack (%)	352:207	21:13	0.89
Geometric mean			
Parasite count/μl	7,079	8,128	0.57
Min-max	(21-105,450)	(986-61,800)	
Hepatomegaly (%)	144:415	4:30	0.07
Splenomegaly (%)	81:478	2:32	0.12
Laboratory data (mean±SD)			
Hematocrit (%)	36.2±6.3	35.6±6.8	0.62
WBC count (per μl)(median)	6.80	7.15	0.15
BUN (mg%)	15.2±6.0	15.3±4.3	0.91
Creatinine (mg%)	1.0±0.3	1.0±0.2	0.79
Total bilirubin (mg%)	1.6±1.0	1.8±1.0	0.14
Albumin (mg%)	4.0±0.4	4.0±0.4	0.83
Globulin (mg%)	3.0±0.5	3.0±0.40	0.89
SGOT (IU/l)	36.6±35.5	32.7±20.2	0.53
SGPT (IU/l)	30.5±40.5	24.7±16.6	0.59

All patients were responsive to chloroquine treatment, which cleared their parasitemia within 7 days; the cure rates were 100% in both groups (Table 2). Parasite clearance time was comparable (59.4±17.5 and 59.8±15.0 hours in G6PD-normal and G6PD-deficient groups respectively; p=0.91). Although fever clearance time was significantly longer in the G6PD-deficient group (45.2±35.2 and 28.0±22.2 hours in G6PD-deficient and G6PD-normal groups, respectively; p<0.01).

DISCUSSION

The treatment of malaria caused by *Plasmodium vivax* has two objectives: to cure the clinical symptoms and to prevent their relapse. Multiple dosage of primaquine (8-aminoquinoline, which is the only drug capable of eliminating persistent liver forms, was given after a full standard-dose course of chloroquine with the aim of preventing relapse after radical treatment of the blood stages. This study showed a 100% cure rate at 28-day follow-up, however there was an approximately 80% cure rate in the previous studies (Bunnag *et al*, 1994; Dohety*et al*, 1997). Since we did not follow-up the patients for longer than 28 days and the tropical strains of *Plasmodium vivax* treated with chloroquine alone may begin 'relapsing' within 5-6 weeks of therapy due to unaffected liver stages of the parasite (hypnozoites) (Collin and Jeffery, 1996), the number of the patients

Table 2
Clinical responses of both G6PD-normal and G6PD-deficient patients.

	G6PD-normal (n=342)	G6PD-deficient (n=22)	p-value
No. of patients withdrawal	217	12	
No. of patients with 28-day follow-up (evaluable pateints)	342	22	
No. of patients cured at day 28	342:342	22:22	1.00
Parasite clearance time (hours)			
Mean±SD	59.4±17.5	59.8±15.0	0.91
Min-max	(20-148)	(33-88)	
Fever clearance time (hours)			
Mean±SD	28.0±22.2	45.2±35.2	<0.01
Min-max	(2-112)	(4-140)	
Blood transfusion	3:339	0:22	0.83
Hct day 0			
Mean±SD	35.8±6.2	35.6±5.1	0.89
Min-max	(14-51)	(22-42)	
Hct day 7			
Mean±SD	34.8±5.0	26.7±5.4	<0.01
Min-max	(19-48)	(20-39)	
Hct day 14			
Mean±SD	35.6±4.3	30.9±3.1	<0.01
Min-max	(24-47)	(26-38)	
Hct day 21			
Mean±SD	37.4±4.0	36.4±2.7	0.30
Min-max	(23-50)	(32-42)	
Hct day 28			
Mean±SD	38.7±4.21	40.8±2.6	0.02
Min-max	(23-50)	(37-46)	

Table 3
Percent reduction in hematocrit.

Percent Reduction in hematocrit	G6PD-normal (n=342)	G6PD-deficient (n=22)	p-value
Day 7			
Mean±SD	(-1.2)±14.4	(-24.5) ±13.9	<0.01
Min-max	(-34.7)-86.7	(-44.7)-4.5	
Day 14			
Mean±SD	1.6±17.8	(-11.0) ±19.3	<0.01
Min-max	(-36.7)-121.4	(-33.3)-50.0	
Day 21			
Mean±SD	7.1±21.4	4.5±17.4	0.57
Min-max	(-30.6)-171.4	(-17.9)-50.0	
Day 28			
Mean±SD	11.2±24.5	17.0±20.1	0.28
Min-max	(-27.3)-192.8	(-4.9)-72.7	

who had relapsed after day 28 was not reported in this study: this relapse rate probably had no significant difference between G6PD-normal and G6PD-deficient patients because the metabolism of primaquine is not influenced by G6PD status (Na Bangchang *et al*, 1994).

A protective mutation against malarial infections is another interesting effect in G6PD-deficient which remains controversial. Some previous studies detected a lower parasite count in G6PD-deficient patients (Allison and Clyde, 1961; Gilles *et al*, 1967; Bienzle *et al*, 1972; Luzzato and Bienzle, 1979) but this was not seen in this study.

Although primaquine is necessary for preventing relapse from *Plasmodium vivax* and *Plasmodium ovale* infections, it can induce acute intravascular hemolysis especially in people with erythrocytic G6PD-deficient (Kruatrachue *et al*, 1962; Benjapongse, 1966; Panich, 1973; Khoo, 1981). Previous studies reported serious hemolysis in these patients (Alving *et al*, 1960), when a course of 15 mg primaquine daily for 14 days was administered. The results of this study revealed that there were significant decreases in the hematocrit levels and increases in the percent reduction of hematocrit levels on day 7 and day 14 in G6PD-deficient patients. Similar to Lederer *et al* (1988), the clinical signs and symptoms of anemia in the G6PD-deficient group seemed to be mild and blood transfusion was not required. This corresponds with the fact that, firstly, there was a high degree of biological, genetic and clinical variation in the presentation of G6PD deficiency (Martin, 1980); secondly, hemoglobinuria did not frequently occur in malaria patients with G6PD *Mahidol*, which is the most common variant in Thailand (Panich *et al*, 1972; Panich, 1973); thirdly, drug-induced hemolysis, which mainly affected old red blood cells with a low G6PD activity, was usually self-limited and stopped before the end of drug administration (Dern *et al*, 1954; Charoenlarp *et al*, 1973). Another study from Myanmar demonstrated that 45 mg primaquine given weekly for 8 weeks was safe for treating *Plasmodium vivax* infection (Myat-Phone-Kyaw *et al*, 1994).

In conclusion, a standard regimen of 1,500 mg chloroquine phosphate over 3 days followed by primaquine 15 mg daily for 14 days for treating *Plasmodium vivax* malaria could be prescribed safely even to those who were G6PD-deficient. In Thailand, especially in the remote areas where a test for G6PD activity is not available, out-patients could have chloroquine and primaquine prescribed simultaneously.

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