PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AMONG VIVAX MALARIA PATIENTS AT THE HOSPITAL FOR TROPICAL DISEASES, THAILAND

Bou Bonito[1], Polrat Wilairatana[1], Noppadon Tangpukdee[1], Sant Muangnoicharoen[1], Kittiyod Poovorawan[1] and Srivicha Krudsood[2]

[1]Department of Clinical Tropical Medicine, [2]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. Glucose-6-phosphate dehydrogenase (G6PD) deficiency and *Plasmodium vivax* malaria infections are not uncommon in Thailand. Primaquine is prescribed to prevent malaria relapse in patients with *P. vivax* malaria but may cause hemolysis in patients with G6PD deficiency. The aim of this study was to determine the prevalence of G6PD deficiency among *P. vivax* malaria patients treated at the Hospital for Tropical Diseases, Bangkok, Thailand during 2005-2014. A population prevalence of G6PD deficiency > 10% warrants screening all *P. vivax* malaria patients for G6PD deficiency prior to prescribing primaquine but a prevalence < 10% may not warrant screening. The results of this study can inform the Ministry of Public Health, Thailand to enable them to make an informed decision when updating the Thai National Treatment Guidelines for *P. vivax* malaria. The study hospital is a referral hospital for malaria treatment in Bangkok. The medical records of all 309 adult patients with *P. vivax* malaria mono-infection treated at the Hospital for Tropical Diseases, Bangkok, Thailand during 2005-2014 were retrospectively reviewed. Malaria was diagnosed by light microscopy. G6PD status was diagnosed by fluorescent spot test. Descriptive data are presented as means and medians. Continuous data with abnormal distribution are presented as medians and interquartile ranges (IQR). Binary outcome data are presented as proportions with 95% confident intervals (95%CI). The prevalence of G6PD deficiency was calculated using period prevalence as the proportion of the study population with an abnormal G6PD screening test during the study period. The chi-square test and odds ratio were used to compare the distribution of categorical variables among the different groups. Of the 309 study subjects, 288 (93.2%) were males. The median (IQR) age of the study subjects was 24 (20-31) years. Sixty-seven point three percent of subjects were Myanmar citizens. Eeleven point five percent of male subjects and 4.8% of female subjects had a positive screening test for G6PD deficiency ($p = 0.48$). Further study using molecular method to detect G6PD deficiency is suggested to better determine the better prevalence of G6PD deficiency in female subjects. Since the prevalence of G6PD deficiency in this study was high (more than 10%) particularly in male patients, the G6PD test should be conducted in all *P. vivax* malaria patients, rather than optional screening test, and should be recommended in the near future Thai National Treatment Guidelines for *P. vivax* malaria in order to avoid hemolysis risk of daily primaquine treatment in the G6PD deficient patients.

Keywords: G6PD, deficiency, vivax, malaria, Thailand

Correspondence: Polrat Wilairatana, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand.
Tel: +66(0) 2354 9100; Fax: +66(0) 2354 9168
E-mail: polrat.wil@mahidol.ac.th

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is an important enzyme in glycolysis in the pentose phosphate pathway in red blood cells (RBCs) (Luzzatto, 2006). G6PD plays an important role in catalyzing Nicotinamide Adenine Dinucleotide Phosphate (NADPH), a coenzyme that decreases glutathione to protect RBCs from oxidation (Cappellini and Fiorelli, 2008). G6PD deficiency can reduce energy in RBCs and may result in RBC hemolysis (Nkhoma *et al*, 2007). The severity of hemolysis depends on the quantity of G6PD, the variant of G6PD deficiency and the nature of the hemolytic agent (Lanzkowsky, 1995).

G6PD deficiency is a hemolytic disorder that occurs in humans (Nkhoma *et al*, 2007) that is an X-linked chromosome defect (Beutler, 1994). G6PD deficiency may have a variety of clinical manifestations, such as chronic non-spherocytic hemolytic anemia, favism, drug-induced acute hemolytic anemia, infection associated hemolytic anemia and neonatal jaundice (Beutler *et al*, 1968). Since G6PD occurs due to an X-chromosome defect, females may have normal, intermediate or deficient (homozygous) phenotypes while males have either normal G6PD or have G6PD deficiency (Luzzatto, 2006). The proportion of those with G6PD deficiency is higher in males than in females (Nance, 1964).

More than 180 mutations of G6PD deficiency have been identified (Minucci *et al*, 2012). G6PD deficiency variants have been reported among various populations in Southeast Asia (Howes *et al*, 2012). G6PD Mahidol is also predominant in Myanmar (Iwai *et al*, 2001). G6PD Viangchan has been reported to be the most common variant in Laotians, Cambodians, Vietnameses and Malaysians (Yusoff, 2002; Ainoon *et al*, 2003; Louicharoen and Nuchprayoon, 2005; Matsuoka *et al*, 2007). One study (Khim *et al*, 2013) from Cambodia found the prevalences of G6PD deficiency among males and females to be 13-26% and 3-4%, respectively. A study from Myanmar among 24 females and 2 males (Matsuoka *et al*, 2004) found 26 G6PD mutations, G6PD Mahidol was found to be 96%, followed by G6PD Union (4%), and G6PD Canton (2.5%). In the Thai population, the prevalences of most common G6PD variants have been reported to be 38.1% in the Mahidol variant, 19.0% in the Viangchan variant, 14.3% in the Chinese-4 variant, 9.5% in the Canton variant, 9.5% in the Union variant, 4.8% in the Kaiping variant and 4.8% in the Gaohe varint (Phompradit *et al*, 2011).

G6PD deficiency overlaps with malaria endemicity supporting the protection against malaria hypothesis (Hedrick, 2011). However, a recent study (Mbanefo *et al*, 2017) found no association between G6PD deficiency and *P. vivax* infection.

The National Guidelines for Malaria Treatment in Thailand recommend giving primaquine daily for 14 days to all *P. vivax* malaria patients except pregnant women and patients with G6PD deficiency. In

patients with G6PD deficiency, administration of a weekly dose of primaquine at a dose of 0.75 mg/kg for 8 weeks is recommended to prevent relapse. Unfortunately none of the malaria clinics along Thai international borders and many small hospitals in Thailand can not screen for G6PD deficiency. *P. vivax* malaria patients are advised by health officers to take daily primaquine for 14 days and if they develop dark urine or anemia it could be due to primaquine-induced hemolysis and they should go to a larger hospital for diagnosis and management. This kind of advice may be inappropriate for the patient but there is no way of knowing the risk for hemolysis in the population most affected by *P. vivax* infection without testing this population for the prevalence of G6PD deficiency.

In this study we aimed to determine the prevalence of G6PD deficiency among *P. vivax* malaria patients treated at the Hospital for Tropical Diseases (HTD) during 2005-2014 to inform the Ministry of Public Health, Thailand to enable them to make an informed decision when updating the Thai National Treatment Guidelines for *P. vivax* malaria. A G6PD prevalence rate >10% would warrant recommending screening *P. vivax* malaria patients for G6PD deficiency prior to prescribing primaquine; however, a rate <10% might recommend against screening for G6PD deficiency.

MATERIALS AND METHODS

This study was conducted at the HTD, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The HTD is a referral hospital for malaria treatment in Bangkok. The medical records of all adult patients presenting to the study hospital during January 2005-December 2014 who were microscopically confirmed to have *P. vivax* mono-infection and who had G6PD testing performed were retrospectively reviewed. The test used to examine for G6PD deficiency was a fluorescent spot test (FST) sometimes called a Beutler test (R&D Diagnostic, Athens, Greece). All study subjects were treated following the Thai National Guidelines for treatment of malaria (Malaria Division, 1996; Bureau of Vector-Borne Diseases, 2006; Royal College of Physicians of Thailand and Department of Disease Control, 2014).

The Kolmogorov-Smirnov goodness-of-fit test was used to test continuous variables for normal distribution of data. Categorical data are presented as percentages and frequencies. Descriptive data are presented as means and medians. Continuous data with abnormal distribution are presented as medians and interquartile ranges (IQR). Binary outcome data are presented as proportions with 95% confidence intervals (95%CI). The prevalence of G6PD deficiency was calculated using period prevalence as the proportion of the study population with an abnormal G6PD screening test during the study period. The chi-square test and odds ratios (OR) were used to compare the distribution of categorical variables among the different groups. Statistical significance was set at $p<0.05$.

This study was approved by Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand (MUTM 2015-054-01).

RESULTS

A total of 309 subjects were included in the study (93.2% males). The median (IQR) age of study subjects was 24 (20-31) years. Sixty-eight percent of subjects came from Tak Province, 11.3% from Kan-

Table 1
Selected demographics of study subjects (*n*=309).

Demography	*n* (%)
Gender	
Male	288 (93.2)
Female	21 (6.8)
Age in years	
15-20	99 (32.0)
21-30	129 (41.8)
31-40	55 (17.8)
41-50	22 (7.1)
≥ 51	4 (1.3)
Ethnicity	
Thai	31 (10.0)
Myanmar	208 (67.3)
Laotian	12 (3.9)
Cambodian	12 (3.9)
Karen	33 (10.7)
Mon	13 (4.2)

chanaburi Province, 2.3% from Si Sa Ket Province and 4.5% from other provinces in Thailand; 67.3 % of subjects came from Myanmar (Table 1).

Of the 309 study subjects, 34 (11%) had G6PD deficiency (Table 2).

DISCUSSION

In this study, the overall prevalence of G6PD deficiency among *P. vivax* malaria patients at the HTD was 11% (11.5% in males and 4.8% in females). These results are similar to a study among newborns at King Chulalongkorn University Hospital, Bangkok, Thailand (11.1% in males and 5.8% in females) (Nuchprayoon *et al*, 2002). Another study from northern Thailand reported higher G6PD deficiency prevalence rates (17% in males and 15% in females) (Pimlak *et al*, 2014).

Table 2
Laboratory results of study subjects (*n*=309) by presence and absence of G6PD findings related to G6PD status.

Tests	G6PD sufficient		G6PD deficient		*p*-value
	n	Median (IQR)	*n*	Median (IQR)	
WBC $x10^3/mm^3$	275	6.16 (5-7.30)	34	5.30 (4.37-6.80)	0.05
RBC $x10^6/mm^3$	275	4.80 (4.50-5.30)	34	4.70 (4.17-5.12)	0.08
Hb g/dl	275	13 (12-13.90)	34	12.30 (11.12-13.75)	0.13
HCT %	275	39.10 (36.20-41.70)	34	37.05 (34.02-43)	0.48
Reticulocytes %	73	1.20 (0.90-1.50)	9	2.90 (1.35-4)	<0.01
TB mg/dl	264	0.80 (0.60-1.47)	31	1.30 (0.80-2.20)	0.01
DB mg/dl	264	0.30 (0.20-0.50)	31	0.40 (0.20-0.70)	0.07
P. vivax count (range)	275	11,880 (3,890-22,000)	34	10,620 (3,067-14,767)	0.21
PCT in hours	275	56 (44-69	34	57 (45-60	0.72
FCT in hours	275	24 (12-32)	34	21 (12-28)	0.24

IQR, interquartile range (Q_1-Q_3); WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; TB, total bilirubin; DB, direct bilirubiin, PCT, parasite clearance time; FCT, fever clearnce time; G6PD, blucose-6-phosphate dehydrogenase.

In our study, the prevalence of G6PD was higher among males than females as would be expected with an X-linked recessive chromosome disorder (Ntaios *et al*, 2008). The test used in this study to diagnose G6PD deficiency has a relatively high false positive rate among women with heterozygous G6PD deficiency (Thielemans *et al*, 2018). Therefore prevalence of G6PD deficiency may be undetected in our study. A future study should be performed using a molecular method to detect G6PD deficiency and better determine the prevalence of G6PD deficiency in female subjects.

In our study of *P. vivax* patients, the prevalence of G6PD deficiency in male subjects was 11.5% and in female subjects was 4.8% and the overall prevalence was 11%. Since it is recommended to screen for G6PD in populations where the overall prevalence of G6PD deficiency is >10%, we conclude all *P. vivax* malaria patients at the study institution should be screened for G6PD deficiency prior to being prescribed primaquine.

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