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Characterization of G6PD genotypes in G6PD deficiency patients from Suratthani Hospital, Thailand

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ABSTRACT

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is highly prevalent in Southeast Asia especially in the area of malaria endemic regions. Primaquine is used for the radical treatment of malaria. However, it causes hemolytic anemia in G6PD deficient patients.

Objectives: To characterize G6PD mutations in G6PD deficient patients around Surat Thani province which is one of the malaria-endemic areas in Thailand.

Materials and methods: One hundred and seventeen leftovered EDTA blood samples were received from primary hospital in Surat Thani Province. All the samples were evaluated for their hematological profiles using an automated hematological analyzer (Beckman Coulter LH 780 Analyzer) and genotyped for G6PD variants using the DiaPlexC G6PD Genotyping Kit.

Results: G6PD mutations were identified in 117 cases of G6PD deficient cases. G6PD Viangchan (51.1%) was identified in 43 Thais and 4 Myanmars, G6PD Mahidol (30.5%) in 16 Thais and 12 Myanmars, G6PD Canton (7.6%) in 4 Thais, 2 Myanmars and 1 Laos, G6PD Kaiping and G6PD Union (4.3% each) in 4 Thais each, and G6PD Mediterranean (2.2%) in 2 Thais. However, the G6PD mutations could not be identified in the remaining 14 samples. There was no evidence of significant differences between hematological parameters among different groups of G6PD variants.

Conclusion: Our study revealed various distribution of G6PD variants in the region, raising the awareness about the requirement for optimal dosage of primaquine in the treatment of malaria infection based on data on G6PD variants.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy which affects around ~400 million people worldwide, particularly in the malaria endemic area such as Asia, Africa, Mediterranean and the Middle East.[1] The G6PD plays a major role in the pentose phosphate pathway by catalyzing the production of nicotinamide adenine dinucleotide phosphate (NADPH). Cells require NADPH for maintaining the effective redox potential that helps against oxidative stress, the mechanism particularly important in red cells.[2] The *G6PD* gene is located on the X-chromosome (Xq28), consisting 13 exons and 12 introns. It encodes 515 amino acids of G6PD enzyme.[3] The mutations in *G6PD* gene usually result in a decrease of the G6PD enzyme in red cells which causes increased susceptibility to oxidative stress. Many drugs and chemicals trigger the oxidative stress in G6PD deficient person leading to hemolytic anemia, neonatal hyperbilirubinemia with kernicterus, chronic non-spherocytic hemolytic anemia (CNSHA), and

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spontaneous abortions.[4] The clinical severity depends on the level of enzyme deficiency (or type of G6PD variant), dose and duration of exposure to the oxidative agent, host factors such as age, level of hemoglobin and concurrent infection.[5,6]

Over 400 G6PD variants have been described based on biochemical properties, and approximately 186 mutations have been identified at the DNA level.[7,8] In Southeast Asia, several G6PD variants have been recognized. For instance, G6PD Surabaya (1291 G>A, Class II) is found in Indonesian Chinese, G6PD Mahidol (487 G>A, Class III) in Myanmar and Thai[9], G6PD Vanua Lava (383 T>C, Class II) in Amboinese, G6PD Viangchan (871 G>A, Class II) in Laos, and G6PD Coimbra (592 C>T, Class II) in Orang Asli.[10] Other mutations such as G6PD Gaohe, (95 A>G, Class III), G6PD Chatham (1003 G>A, Class II), G6PD Union (1360 C>T, Class II), G6PD Canton (1376 G>T, Class II), G6PD Chinese 4 (392 G>T, Class III), and G6PD Kaiping (1388 G>A, Class II) have also been reported. In Thailand, G6PD Viangchan variant is highly prevalent and predominates in the eastern part of the country while the Mahidol variant is obsessed in the western part of the country.[11-12] Other G6PD variants such as G6PD Kaiping, G6PD Union, G6PD Canton, G6PD Gaohe are found in Chinese, Indian and certain areas in Southeast Asia.

Since the early 1950s, primaquine has been the only drug to eliminate hypnozoidal for *P. vivax* and *P. ovale* and potent gametocytocide for *P. falciparum*.[13] However, it could cause dose-dependent, severe hemolysis for G6PD deficient person.[14] In recent years, Surat Thani Province is ranked one of top eight provinces in Thailand with high incidence of malaria.[15] The province is covered with rain forests, and rubber and palm oil plantations, making a good ground for malaria transmission.

In this work, we aimed to study the characteristics of glucose-6-phosphate dehydrogenase variants among G6PD patients around Surat Thani Province, Thailand.

Materials and methods

Sample collection

One hundred and seventeen leftovered EDTA blood samples were positive for routine G6PD fluorescent screening test done by Hematology Unit, Suratthani hospital, between March 2016 and November 2016. These samples were sent from the primary hospitals in Surat Thani Province which included Kanchanadit Hospital, Tha Rong Chang Hospital, Phanom Hospital, Khian Sa Hospital, Chaiya Hospital, Phunphin Hospital and Suratthani Hospital for routine G6PD screening test. All blood samples were evaluated for their hematological profiles using an automated hematological analyzer (Beckman Coulter LH 780 Analyzer). Other common characteristics such as age, gender, and nationality were also collected. The study protocol was approved by the ethical committee of Suratthani Hospital. (EC code 11/2560). For all the samples used in this study, written informed consent was obtained from the participants or from their parents.

Fluorescent spot test

Fluorescent spot test, a routine test for G6PD deficiency, was performed immediately after receiving the blood sample from the primary hospitals using the R&D G6PD kit (R&D Diagnosis, Greece) according to the manufacturer's recommendations (catalog number SQMMR500).[16] Briefly, 5 µL of EDTA blood was mixed with 100 µL of reagents (containing 1mmol/L of G6PD-6-phosphate, 0.75 mmol/L of NADP, 0.8 mmol/L of GSSG (oxidized glutathione), 0.2% of Saponin and 225 mmol/L, pH 7.8 of Tris (hydroxymethyl)-aminomethane). After 10 min of incubation at room temperature, 15 µL aliquot was spotted on filter paper and allowed to air dry. The spots were then visualized under UV light. The normal G6PD activity showed a fluorescence spot while no fluorescence signals G6PD deficiency.

Hematological data

Hematological data were collected from routine Hematology unit, comprising WBC count, RBC count, Hemoglobin (Hb) level, Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC). The Beckman Coulter LH 750 Hematology Analyzer (Beckman Coulter, Inc, Miami, FL, USA) was used to generate these hematological data.

Detection of G6PD variants

DNA was extracted from 200 µL of individual blood sample using a NucleoSpin blood kit (Macherey-Nagel, Duren, Germany) and eluted in 100 µL of BE buffer. DNA was kept at -20°C until genotyping was performed. G6PD variants were detected using the DiaPlexC G6PD Genotyping Kit (Asian type; SolGent, ROK). The kit employed one-step multi-allelic specific PCR to detect 8 common mutations of G6PD frequently found in Asia. The 8 variants generated PCR products of different amplicons based on the G6PD mutation types: Vanua Lava (383 T>C, 154 bp), Mediterranean (563 C>T, 262 bp), Coimbra (592 C>T, 234 bp), Mahidol (487 G>A, 337 bp), Viangchan (871 G>A, 501 bp), Kaiping (1388 G>A, 557 bp), Canton (1376 G>T, 681 bp) and Union (1360 C>T, 803 bp). The PCR reactions were: initial denaturation at 95°C for 15 min; 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 40 s; and a final extension at 72°C for 5 min. 25 µL of each PCR mixture contained 5 µL of template DNA (25–50 ng or 100 ng), 12.5 µL of 2X multiplex PCR smart mix (G6PD Asian type), 2 µL of primer mixer (G6PD Asian type), 5.5 µL of nuclease free water. The PCR products were visualized using 3% agarose gel. The internal control of each PCR reaction was confirmed at band 1234 bp.

Data analysis

Data analysis was performed using the SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL, USA). The descriptive statistics include the number and percentage of the distribution of the G6PD variants according to nationalities. One-way ANOVA or unpaired Student t-test was used to assess the mean difference of the hematological characteristics among G6PD variants. The statistical significance was considered at p-value of less than 0.05.

Results

In this study, we characterized the G6PD variants from the positive routine G6PD fluorescent screening test samples obtained from hospitals in the malaria risk area of Surat Thani Province. A total of 117 samples comprised 96 newborns (mean 5 days), 10 infants (mean 10 months), 4 children (mean 4.3 years) and 7 adults (mean 46 years). 93 subjects (79.5%) were males of Thai nationality (76 newborns, 8 infants, 3 children and 6 adults), 23 subjects (19.66%) (22 males and 1 female) were Myanmar (20 newborns, 2 infants and 1 adult), and 1 Laos (0.84%) male newborn. The most common variants detected were G6PD Viangchan (51.1%), which was observed in 43 Thais and 4 Myanmars (Table 1). The second most common was G6PD Mahidol (30.5%), found in 16 Thais and 12 Myanmars. G6PD Canton (7.6%) was detected in 4 Thais, 2 Myanmars and one Laos, while G6PD Kaiping (4.3%) and G6PD Union (4.3%) was found in 4 Thais each. Two other cases were G6PD Mediterranean (2.2%) and they belonged to Thais. The agarose gel detection of the G6PD variants was shown in Figure 1. The 14 samples could not be identified for G6PD variants and 11 other samples had no amplified PCR product due to the low concentration of genomic DNA.

To study the hematological effects of the different G6PD mutations, the hematological parameters were compared in each age groups. We categorized the subjects into four different age groups: newborns (NB, <30 days), infants (IF, 1-12 months), children (CH, 1-12 years) and adults (AD, >12 years). There was no significant difference between the parameters among different G6PD variants in each age category (Tables 2, 3). In our study, six patients had anemia with Hb level of less than 11 g/dL, and Hct levels less than 30.4%: (1) the eight months old female patient with Viangchan mutation, Hb level decreased to 2.8 g/dl, and Hct was 7.6%; (2) two infants males (1 month) with Viangchan mutation with Hb level of 7.8 and 9 g/dl, and Hct value was 22.8 and 27.3% respectively; (3) two adult males of 40 and 74 years old with Mahidol mutation, Hb values of 7.6 and 9.4 g/dl respectively, and Hct level of 12.8 and 26.7%; (4) one male child (2 years) with Union mutation, Hb value of 4.6 g/dl and Hct 12.8%.

Table 1 G6PD variants according to ethnic group in Surat Thani Province.

G6PD variants*	Thai [n (%)]	Myanmar [n (%)]	Laos [n (%)]	Total [n(%)]
Viangchan (871 G>A)	43 (46.8%)	4 (4.3%)	-	47 (51.1%)
Mahidol (487 G>A)	16 (17.4%)	12 (13.1%)	-	28 (30.5%)
Canton (1376 G>T)	4 (4.3%)	2 (2.2%)	1 (1.1%)	7 (7.6%)
Kaiping (1388 G>A)	4 (4.3%)	-	-	4 (4.3%)
Union (1360 C>T)	4 (4.3%)	-	-	4 (4.3%)
Mediterranean (563 C>T)	2 (2.2%)	-	-	2 (2.2%)
Vanua Lava (383 T>C)	Not found			
Coimbra (592 C>T)	Not found			
Total	73 (79.3%)	18 (19.6)	1 (1.1%)	92 (100%)

**Detected by DiaPlexC G6PD Genotyping Kit (Asian type) which could detect 8 G6PD variants.*

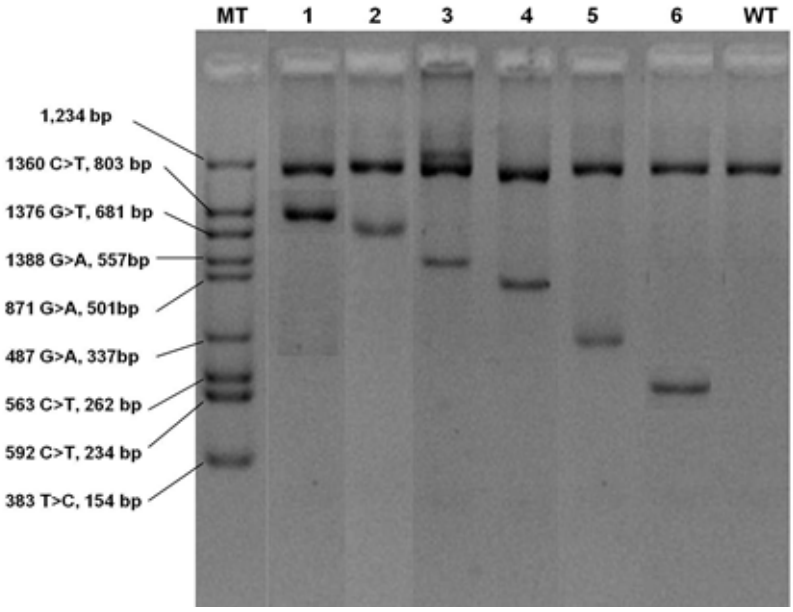

Figure 1. *Agarose gel detection of the G6PD variants using the G6PD genotyping kit (Asian type, DiaPlexC). MT: Mutant type control, 1: Union mutant, 2: Canton mutant, 3: Kaiping mutant, 4: Viangchan mutant, 5: Mahidol mutant, 6: Mediterranean mutant, WT: Wild type control.*

Table 2 Comparison of Rbc, Hb and Hct and G6PD genotypes with the respect in age group.

Parameter	Age	G6PD genotypes														*P* value
		Viangchan		Mahidol		Union		Canton		Kaiping		Mediterranean		Unknown		
		No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	
Rbc, 10^{12}/L	NB	37	4.5±0.7	24	4.7±0.7	3	4.9±0.5	7	4.4±0.7	14	4.4±0.5	1	4.04	11	4.6±0.7	0.555[a]
	IF	6	3.2±1.4	2	3.5±0.4	0	0	0	0	0	0	1	3.67	1	5.44	0.610[b]
	CH	2	4.3±0.2	0	0	1	3.5	0	0	0	0	0	0	0	0	-
	AD	2	4.1±0.1	2	2.0±0.8	0	0	0	0	0	0	0	0	2	2.5±1.8	0.299[a]
Hb, g/dL	NB	37	15.1±2.6	24	15.7±2.5	3	14.2±0.9	7	15.0±2.2	14	13.8±1.4	1	13.9	11	15.1±2.3	0.296[a]
	IF	6	10.2±4.7	2	10.8±0.4	0	0	0	0	0	0	1	16.5	1	12.3	0.629[b]
	CH	2	11.1±0.1	0	0	1	4.6	0	0	0	0	0	0	0	0	-
	AD	2	13.4±1.6	2	7±3.4	0	0	0	0	0	0	0	0	2	11.3±9.3	0.589[a]
Hct, %	NB	37	46.0±8.1	24	48.3±7.9	3	42.9±3.0	7	45.3±6.6	14	41.7±4.0	1	43	11	45.3±8.0	0.191[a]
	IF	6	30.8±15.0	2	32.8±1.8	0	0	0	0	0	0	1	48.3	1	40	0.628[b]
	CH	2	34.2±1.6	0	0	1	12.8	0	0	0	0	0	0	0	0	-
	AD	2	40.7±5.0	2	19.8±9.8	0	0	0	0	0	0	0	0	2	21.9±13.0	0.21[a]

Note: *Newborn (<30 days), IF: Infant (1-12 months), CH: Children (1-12 years), AD: Adult (>12 years).[a] p value according to one-way ANOVA. [b] p value according to unpaired t-test, Rbc: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit*

Table 3 Comparison of MCV, MCH and MCHC and G6PD genotypes with the respect in age group.

Parameter	Age	G6PD genotypes														*P* value
		Viangchan		Mahidol		Union		Canton		Kaiping		Mediterranean		Unknown		
		No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	
Rbc, 10^{12}/L	NB	37	100.2±12.0	24	102.6±5.8	3	96.4±6.5	7	101.5±4.3	14	94.8±12.0	1	106.6	11	96.7±13.5	0.279[a]
	IF	6	92.4±15.2	2	93.9±4.2	0	0	0	0	0	0	1	104	1	70.8	0.930[b]
	CH	2	79.4±0.4	0	0	1	90.4	0	0	0	0	0	0	0	0	-
	AD	2	88.3±6.9	2	94.5±13.0	0	0	0	0	0	0	0	0	2	94.5±11.0	0.809[a]
Hb, g/dL	NB	37	34.6±7.2	24	34.9±8.3	3	38.2±2.7	7	33.4±1.8	14	31.4±4.1	1	34.3	11	31.1±3.9	0.283[a]
	IF	6	31.0±4.0	2	31±2.0	0	0	0	0	0	0	1	35.2	1	22.7	0.920[b]
	CH	2	26.8±1.2	0	0	1	24.5	0	0	0	0	0	0	0	0	-
	AD	2	28.7±2.6	2	33.3±4.2	0	0	0	0	0	0	0	0	2	33.8±5.6	0.510[a]
Hct, %	NB	37	33±1.2	24	32.5±0.8	3	34.5±3.2	7	32.7±0.7	14	33.1±0.3	1	32	11	32.6±1.2	0.069[a]
	IF	6	33.8±1.7	2	33±0.7	0	0	0	0	0	0	1	32	1	32	0.870[b]
	CH	2	33.6±1.6	0	0	1	22.8	0	0	0	0	0	0	0	0	-
	AD	2	32.6±0.3	2	35.3±0.4	0	0	0	0	0	0	0	0	2	55.7±26.6	0.381[a]

NB: Newborn (< 30 days), IF: Infant (1 – 12 months), CH: Children (1 – 12 years), AD: Adult (>12 years). [a] p-value according to one-way ANOVA. [b] p-value according to unpaired t-test (p<0.05). Rbc: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit

Discussion

Mutations in G6PD gene were genotyped using DiaPlexC G6PD Genotyping Kit (Asian type). The method was rapid, highly sensitive and specific[17] and the kit could detect 8 variants: G6PD Vanua Lava, Union, Kaiping, Mahidol, Canton, Coimbra, Mediterranean and Viangchan. Five of these eight variants, G6PD Union, Kaiping, Canton, Coimbra and Mediterranean, can cause severe acute hemolytic anemia as only 1-10% of enzyme activity is present with these variants. Other G6PD mutations i.e. G6PD Viangchan, Canton and Mahidol have 10-60% of enzyme activity remains, causing less severe acute hemolytic anemia.[18]

The most frequent mutations in the present study was G6PD Viangchan which has been previously reported as the common variant in Thais from central part of Thailand, in Cambodian, Laotian who live along the border with Thailand[10-12,19] and in Malay.[20,21] However, G6PD Viangchan was not frequently found in Myanmar.[10] Instead, G6PD Mahidol (487, G>A) was the most common variant observed among Myanmars. This variant could also be found in the central part of Thailand[10], Indonesia, and Malaysia.[20] G6PD Mediterranean (563C>T), which is common in Mediterranean countries and Indo-Pakistan areas, was also detected in 2 Thais.

Surat Thani is a populous province with a significant number of migrant workers from other countries. As it is

one of the malaria endemic provinces in Thailand, the information about the G6PD variants would help in adjusting the dose of anti-malarial drugs. The dosage regimen of primaquine and duration of exposure to the drug for treatment of both *P. falciparum* and *P. vivax* malaria may need to be optimized based on G6PD variants.

Conclusion

Our study focused on the characterization of G6PD variants obtained from hospitals in the malaria risk area of Surat Thani Province. Six G6PD variants would be associated with clinical hemolysis during treatment with primaquine were found. Therefore, our results revealed various distribution of G6PD variants in the region, raising the awareness about the requirement for optimal dosage of primaquine in the treatment of malaria infection based on G6PD variants.

Limitations of the study

There were fourteen samples which could not be identified for G6PD variants due to the limitation of the DiaPlexC G6PD Genotyping Kit (Asian type). The kit is unable to detect a certain variants such as G6PD Quing Yuan (392 G>T), G6PD Songklanagarind 196 T>A, silent mutation (1311 C>T) and G6PD Gaohe (95, A>G). However, the results of this study still provided valuable information regarding diagnosis G6PD variants in southern regions of Thailand.

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