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Prevalence of Glucose 6-Phosphate Dehydrogenase Variants in Malaria-Endemic Areas of South Central Timor, Eastern Indonesia

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Abstract. Primaquine is an effective anti-hypnozoite drug for *Plasmodium vivax* and *Plasmodium ovale.* However, it can trigger erythrocyte hemolysis in people with glucose 6-phosphate dehydrogenase (G6PD) deficiency. In a previous report from South Central Timor (SCT), Indonesia, we described the prevalence of Vanua Lava, Chatham, and Viangchan variants; in this study, other G6PD variants (Kaiping, Coimbra, Gaohe, Canton, and Mahidol) were subsequently analyzed. For clarity, all of these results are described together. The 381 DNA samples from the previous study during 2013–2014 were analyzed for G6PD variants by using PCR-restriction fragment length polymorphism (RFLP). The prevalence of G6PD deficiency in SCT was 6.3% (24/381 cases), including 4.2% (16/381 cases), 0.5% (2/381 cases), and 1.6% (6/381 cases) for Coimbra, Kaiping, and Vanua Lava variants, respectively. No other variants were found in this population. A significant association was found between ethnicity and the distribution of G6PD Kaiping in female subjects. A positive association was shown between G6PD activity and heterozygous females carrying Coimbra genotype, hemizygous males carrying Vanua Lava, *Plasmodium falciparum* infection in female subjects, and *P. vivax* infection in male subjects. Further molecular analysis of heterozygous females, particularly in malaria-endemic areas, is needed for mapping distribution of G6PD deficiency status in Indonesia.

INTRODUCTION

The Ministry of Health of the Republic of Indonesia plans to eliminate malaria by 2030.[1] The malaria incidence in Indonesia has declined from an annual parasite incidence of 1.8% (422,447 cases) in 2011–0.8% (218,450 cases) in 2016.[2] Despite such a decline, there remain problems in achieving malaria elimination in Indonesia. Major obstacles include the emergence of antimalarial drug resistance, mosquito resistance to insecticides, and inadequate health system performance.[3–5] The predominant malaria species are *Plasmodium falciparum* and *Plasmodium vivax*, with prevalences of 62% and 33%, respectively.[6] Among patients hospitalized with a primary diagnosis of malaria, *vivax* and *falciparum* malaria patients are often equally likely not to survive. Severe *P. vivax* infection has been reported from various endemic regions, with a similar risk of death to that of *P. falciparum* infection.[7] Chloroquine-resistant *P. vivax* was first reported in 1989, almost 30 years after chloroquine-resistant *P. falciparum* was first noted. Declining efficacy of chloroquine against *P. vivax* was reported in *P. vivax*–endemic areas of the world, including Indonesia.[8–12] Some countries have, therefore, revised their national antimalarial guidelines to recommend artemisinin-based combination therapy (ACT) for the treatment of both *P. falciparum* and *P. vivax* infections. Currently, the Indonesian Ministry of Health recommends dihydroartemisinin–piperaquine (DHP) in combination with primaquine for radical cure of all *P. vivax* cases.[13]

Primaquine is a potent antimalarial drug for blocking the transmission of *P. falciparum* infection and preventing the relapse of *P. vivax* hypnozoites. Nevertheless, the concern of its clinical use is the risk of intravascular hemolysis, particularly in individuals with glucose 6-phosphate dehydrogenase (G6PD) deficiency. The WHO recommends primaquine at a dose of 0.25–0.5 mg per kg body weight be given daily for 14 days in all *P. vivax* patients with normal G6PD activity.[14] Glucose 6-phosphate dehydrogenase testing is not usually available in most malaria-endemic areas of the world, and, therefore, lower doses of primaquine are recommended to reduce the risk of drug-induced hemolysis.[15,16] In Indonesia, the recommended dose regimen for *P. vivax* patients with normal G6PD activity is 0.25 mg/kg daily for 14 days, with an increase to 0.5 mg/kg daily for 14 days in case of relapse. For those who are suspected of G6PD deficiency, primaquine is given at a dose of 0.75 mg/kg per week for 8–12 weeks.[13,17] Testing for G6PD deficiency before primaquine treatment of patients with *P. vivax* malaria cannot be routinely applied in malaria-endemic areas because of the time-consuming procedure, and the requirement of specialized equipment and laboratory skills.[18] Administration of primaquine without information on G6PD status may lead to an acute hemolytic anemia attack in patients with G6PD deficiency, as well as to increased morbidity from *P. vivax* infection.[19] Mapping of G6PD deficiency variants in malaria-endemic populations is therefore essential to support the Indonesian malaria elimination program.

Glucose 6-phosphate dehydrogenase deficiency is the most common genetic disorder found in Southeast Asia, especially in malaria-endemic areas. It has been claimed to be a protective factor against malaria infection.[20] The gene encoding the G6PD enzyme consists of 1,545 base pairs separated into 13 exons. Almost all types of G6PD deficiency are caused by a single nucleotide mutation, resulting in the change of one amino acid change among a total of 515 amino acids.[21,22] At least 217 mutations in the G6PD gene have been reported around the world.[23] The G6PD gene is located on the X-chromosome, and, therefore, females can carry either homozygous or heterozygous genotypes, but males can only carry hemizygous genotypes.[19]

In Eastern Indonesia, the common reported G6PD variants are Vanua Lava (10884T>C), Viangchan (871G>A), Chatham (1003G>A), Kaiping (1388G>A), and Coimbra

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(592C>T).[21,22,24,25] In a previous report (2013–2014) from South Central Timor (SCT), Indonesia, three G6PD variants (Vanua Lava, Chatham, and Viangchan) were analyzed. The Vanua Lava variant (10884T>C) was the most common variant (6.5%, 6/92 cases), whereas Chatham (1003G>A) and Viangchan (871G>A) variants were absent.[24] To enrich the data on G6PD status in SCT, other G6PD variants (Kaiping, Coimbra, Gaohe, Canton, and Mahidol) were subsequently analyzed in this study. For clarity, all of these results are described together.

MATERIALS AND METHODS

Study design and samples. This study was conducted in Jakarta, Indonesia, from 2018 to 2019. Stored DNA samples collected from the previous study (2013–2014) in the SCT district, Eastern Indonesia,[24] were selected by purposive sampling with background demographic data fulfilling at least one of the following three criteria: 1) asymptomatic malaria (confirmed by PCR), 2) G6PD deficiency (G6PD level < 6.97 U/gHb by Randox test), or 3) anemia (hemoglobin level < 13 g/dL in males and < 11 g/dL in females). A total of 381 DNA samples were finally included in the analysis (from 156 males and 225 females). The background demographic data from the previous study[24] were analyzed to investigate their association with G6PD variant status.

DNA extraction. Archived DNA samples (at −80°C) had previously been extracted from whole blood samples (200 µL each) by using the Nucleon Genomic DNA Extraction kit (Promega, Madison, WI).[24]

Genotyping of G6PD variants. The five common G6PD variants found in Indonesia, that is, Coimbra (592C>T), Gaohe (95A>G), Canton (1376G>T), Mahidol (487G>A), and Kaiping (1388G>A), were analyzed using PCR-RFLP according to the modified methods provided in Table 1. The primer pairs and appropriate restriction enzymes used in the analysis are presented in Table 1. Briefly, PCR mixtures (25 µL) consisted of 2 µL of DNA solution, 1 × buffer with $(NH_4)_2SO_4$, 0.2 µM of each primer, 200 µM each dNTP, 2 mM $MgCl_2$, and 1 U of *Taq* polymerase. The amplification process was performed using a Bio-Rad C1000 thermal cycler with the conditions summarized in Table 1. The PCR product was digested with the restriction enzymes indicated in Table 1. The targeted band was identified under UV light using a Bio-Rad molecular imager, gel doc XR+ (Bio-Rad Laboratories, Inc., Hercules, CA).

Data analysis. Statistical analysis was performed using IBM SPSS statistics for Windows (version 22.0. IBM Corp. Armonk, NY). Results of the G6PD variants from the previous study[24] and the current study were combined to obtain the prevalence of G6PD variants in SCT. The demographic data, G6PD genotypes, and allele frequencies are presented as the number and percentage values. The chi-square test was used to determine the association between G6PD variants and demographic data, as well as the association between G6PD (activity and genotype) and malaria infection. The allele frequency of each mutation variant was calculated using the Hardy–Weinberg equilibrium equation. The statistical significance level was set at $\alpha = 0.05$.

Ethical review board. The study was approved by the Health Research Ethics Committee of the National Institute of Health Research and Development, Indonesia (Ethical approval number LB.02.01/2/KE.334/2018).

RESULTS

Demographic data of the sample population. The demographic data of a total of 381 samples are presented in Table 2. Of 381 samples, 225 (59%) and 156 (41%) samples were obtained from female and male subjects, respectively. The age was grouped according to the WHO guidelines.[26] Three-hundred sixty-five (95.8%) and 16 (4.2%) samples were obtained from adults (> 19 years) and pediatrics (≤ 19 years), respectively. Ethnicity was grouped as Timorese [348 (91.3%) cases] and others, that is, Rotenese (20 cases, 5.2%), Savunese (seven cases, 1.8%), Sumbanese (one case, 0.3%), Bataknese (one case, 0.3%), Alorese (one case, 0.3%), Buginese (one case, 0.3%), Ambonese (one case, 0.3%), and Belunese (one case, 0.3%). Two-hundred twenty-six (59.3%) samples were collected from the northern part of Indonesia: 76 (19.9%), 70 (18.4%), and 80 (21.0%) samples from Oinlasi, Oe'ekam, and Oenino, respectively. One-hundred fifty-five (40.7%) samples were obtained from the southern part: 67 (17.6%) and 88 (23.1%) samples from Panite and Batu Putih, respectively. Two-hundred twenty-five (59.1%) samples were anemic, and 156 (40.9%) samples were non-anemic (samples with normal hemoglobin levels). Based on PCR analysis, asymptomatic cases were detected in 181 (47.5%) samples: 95 (24.9%), 57 (15%), and 29 (7.6%) samples were *P. vivax*, *P. falciparum*, and mixed infection of *P. falciparum* and *P. vivax*, respectively. Based on G6PD screening by the Randox test, 96 (25.2%) and 285 (74.8%) samples were classified as G6PD deficient and G6PD normal, respectively.

Glucose 6-phosphate dehydrogenase variants. By combining the results of the previous study and the current

TABLE 1
PCR-RFLP conditions for the analysis of five variant glucose 6-phosphate dehydrogenase mutations

Primer name	Primer's sequence 5′-3′	Exon	Name of the variant	T (°C) annealing	Amplicon length (bp)	RE	Fragment size (bp)	References
95F	CTC TAG AAA GGG GCT AAC TTC TCA	2	Gaohe (95A>G)	60	198	*Mlu*I	N 198, M 174 + 24	(34,48)
95R	GAT GCA CCC ATG ATG ATG AAT ACG							
487F	GCG TCT GAA TGA TGC AGC TCT GAT	6	Mahidol (487 G>A)	60	104	*Hind*III	N 104, M 82 + 22	(18,48)
487R	CTC CAC GAT GAT GCG GTT CAA GC							
592F	GAG GAG GTT CTG GCC TCT ACT C	6	Coimbra (592C>T)	65	240	*Pst*I	N 157 + 83 M 157 + 63+20	(34,48)
592R	TTG CCC AGG TAG TGG TCG CTG C							
1376F	ACG TGA AGC TCC CTG ACG C	12	Canton (1376 G>T)	62	214	*Afl*II	N 214 M 194 + 20	(34,48)
1376R	GTG AAA ATA CGC CAG GCC TTA							
KP-9F	ACG TGA AGC TCC CTG ACG C	12	Kaiping (1388G>A)	65	227	*Nde*I	N 227 M 206 + 21	(18,34)
KP-9R	GTG CAG CAG TGG GGT GAA CAT A							

N = normal; M = mutation; RE = restriction enzyme.

TABLE 2
Demographic characteristics of the sample population from South Central Timor, Indonesia

	Number of samples (N = 381), n (%)
Age-group (WHO)*	
Pediatrics	16 (4.2)
Adults	365 (95.8)
Gender	
Male	156 (40.9)
Female	225 (59.1)
Ethnicity	
Timorese	348 (91.3)
Others	33 (8.7)
Subdistrict group	
Northern part	226 (59.3)
Southern part	155 (40.7)
Anemia status	
Anemia†	225 (59.1)
Normal	156 (40.9)
Malaria infection (nested PCR)	
P. falciparum	57 (15.0)
P. vivax	95 (24.9)
Mix (*P. falciparum* and *P. vivax*)	29 (7.6)
Noninfected	200 (52.5)
G6PD screening activity (Randox test)‡	
Normal	285 (74.8)
Deficient	96 (25.2)

G6PD = glucose 6-phosphate dehydrogenase; *P. falciparum* = *Plasmodium falciparum*; *P. vivax* = *Plasmodium vivax*.
* Age-group (pediatrics ≤ 19 years and adults > 19 years).
† Anemia (male < 13 g/dL and female < 11 g/dL).
‡ G6PD screening activity (normal > 6.97 U/gHb and deficient ≤ 6.97 U/gHb).

study, there are three G6PD variants found in SCT. Sixteen (4.2%), two (0.5%), and six (1.6%) were G6PD Coimbra, Kaiping, and Vanua Lava variants, respectively. Samples that carried Coimbra and Kaiping G6PD variants were obtained from heterozygous females with allele frequencies 0.036 and 0.004, respectively. Of Vanua Lava G6PD variants, 4, 1, and 1 samples were obtained from hemizygous males, a heterozygous female, and a homozygous female, respectively. The allele frequencies of the Vanua Lava variants were 0.007 in females and 0.026 in males. No samples carried other G6PD variants (Chatham, Viangchan, Gaohe, Canton, and Mahidol variants) (Table 3).

Factors associated with G6PD variants. The analysis of factors that could contribute to the distribution of G6PD is shown in Table 4. The distribution of G6PD Kaiping type in females was associated with ethnicity (P = 0.014). Based on the G6PD screening by the Randox test, G6PD deficiency was associated with heterozygous females having the Coimbra genotype (P = 0.011) and hemizygous males carrying the Vanua Lava variant (P = 0.001). However, normal G6PD was also reported by this test from eight female samples carrying the Coimbra variant, all female samples which have the Kaiping variant, and 1 from the homozygous female with the Vanua Lava variant.

Association between G6PD status and malaria infection. Glucose 6-phosphate dehydrogenase status in female and male subjects by the Randox test was associated with malaria infection (Table 5). In females, individuals with G6PD deficiency were associated with *P. falciparum* infection; the odds of infection were 0.0645 times of those with normal G6PD activity (OR = 0.0645 [95% CI: 0.0085–0.4889] P = 0.008). No association was observed in *P. vivax* or mixed infections. In males, on the other hand, G6PD deficiency was associated with *P. vivax* infection; the odds of *P. vivax* infection was 0.1690 times of those with normal G6PD activity (OR = 0.1690 [95% CI: 0.0548–0.5208] P = 0.002).

DISCUSSION

Malaria is still a major health problem in Indonesia, particularly in Eastern Indonesia. The program to achieve malaria elimination in Indonesia is to be carried out in four stages: 1) The Thousand Islands (Jakarta), and Bali and Batam islands in 2010; 2) Java, Aceh, and the Riau Islands in 2015; 3) Sumatra, West Nusa Tenggara, Kalimantan, and Sulawesi in 2020; and 4) Papua, West Papua, East Nusa Tenggara, and the Maluku Islands in 2030.[1,27] Elimination is expected to be successful with the proper use of antimalarial drugs. The downside of radical treatment recommended by the Indonesian Ministry of Health (DHP combined with primaquine) is that primaquine can be toxic to people with G6PD deficiency.[13,15,17,28,29]

The results from this study were combined with those of the previous study conducted by Hutagalung and others.[24] They showed that Coimbra was the most dominant G6PD variant in the SCT region, followed by Vanua Lava and Kaiping variants. The Vanua Lava variant was the dominant G6PD variant in Eastern Indonesia.[18,21,22,25,30] A study by Kawamoto et al.[31] reported that Kaiping and Coimbra mutation variants were prevalent on Flores Island, Eastern Indonesia, with frequencies of 50% and 9.1%, respectively, among people of Sikka ethnicity. The prevalence of the Kaiping variant was 14.3% among members of Ende ethnicity, and the prevalence of the Coimbra variant was 5.6% among members of Bajo ethnicity. These two variants were also reported on Flores Island, with the frequency of 1.4% for the Coimbra variant, 0.8% for Kaiping together with Vanua Lava, Viangchan, and Chatham variants, each with a frequency of 0.3%.[21] In another study in Eastern Indonesia, 11% (5/44 cases) Coimbra variant

TABLE 3
Prevalence and allelic frequency of G6PD variants in South Central Timor, Indonesia

Variant	Nucleotide substitution	Amino acid substitution	Ref SNP ID	Class	Number of samples, n (%)	Allele frequency	Female: male ratio
Coimbra	592C>T	198 Arg > Cys	rs137852330	2	16 (4.2)	0.036 (female)	16:0
Kaiping	1388G>A	463 Arg > His	rs72554664	2	2 (0.5)	0.004 (female)	2:0
Gaohe	95A>G	32 His > Arg	rs137852340	3	0 (0)	–	–
Mahidol	487G>A	163 Gly > Ser	rs137852314	3	0 (0)	–	–
Canton	1376G>T	459 Arg > Leu	rs72554665	3	0 (0)	–	–
Vanua Lava*	10884T>C	128 Leu > Pro	rs78365220	2	6 (1.6)	0.007 (female), 0.026 (male)	2:4
Chatham*	1003G>A	335 Ala > Thr	rs5030869	2	0 (0)	–	–
Viangchan*	871G>A	291 Val > Met	rs137852327	3	0 (0)	–	–

G6PD = glucose 6-phosphate dehydrogenase.
* G6PD variants were investigated in the previous study.[24]

TABLE 4
Factors associated with G6PD variants in female and male members of the South Central Timor population

	G6PD variant, *n* (%)						
	Female (*n* = 225 [59.1%])					Male (*n* = 156 [40.9%])	
	No mutation	Coimbra,* heterozygous	Kaiping,* heterozygous	Vanua Lava,† heterozygous	Vanua Lava,† homozygous	No mutation	Vanua Lava,† hemizygous
Age-group (WHO)‡							
Pediatrics	6 (1.6)	–	–	–	–	10 (2.6)	–
Adults	199 (52.2)	16 (4.2)	2 (0.5)	1 (0.3)	1 (0.3)	142 (37.3)	4 (1.0)
Ethnicity							
Timorese	192 (50.3)	15 (3.9)	1 (0.3)	1 (0.3)	1 (0.3)	134 (35.2)	4 (1.0)
Others	13 (3.4)	1 (0.3)	1 (0.3) (P = 0.014)	–	–	18 (4.7)	–
Subdistrict group							
Northern part	129 (33.9)	13 (3.4)	–	–	–	83 (21.7)	1 (0.3)
Southern part	76 (19.9)	3 (0.8)	2 (0.5)	1 (0.3)	1 (0.3)	69 (18.1)	3 (0.8)
Anemia status							
Normal	85 (22.3)	10 (2.6)	–	1 (0.3)	1 (0.3)	56 (14.7)	3 (0.8)
Anemia§	120 (31.5)	6 (1.6)	2 (0.5)	–	–	96 (25.1)	1 (0.3)
G6PD screening activity (Randox test)‖							
Normal	160 (42.0)	8 (2.1)	2 (0.5)	–	1 (0.3)	114 (29.9)	–
Deficient	45 (11.8)	8 (2.1) (P = 0.011)	–	1 (0.3)	–	38 (10.0)	4 (1.0) (P = 0.001)

G6PD = glucose 6-phosphate dehydrogenase.
*, All carrier mutation samples were heterozygous female; thus, male hemizygous allele (TT for Coimbra and AA for Kaiping) are not listed in the table.
† Data from the previous study.[24]
‡ Age-group (pediatrics ≤ 19 years and adults > 19 years).
§ Anemia (male < 13 g/dL and female < 11 g/dL).
‖ G6PD screening activity (normal > 6.97 U/gHb and deficient ≤ 6.97 U/gHb).

frequency was reported among villagers of Panenggo Ede.[18] A common variant in Asia, the Mahidol variant, was rarely reported in Java, West Indonesia.[32] The G6PD Mahidol variant has been reported as a dominant G6PD mutation variant in the Thailand–Myanmar border population.[33,34]

Glucose 6-phosphate dehydrogenase Coimbra and Kaiping variants were classified as class II G6PD variants based on the WHO guidelines (class I, the most severe–class V, the mildest).[35] The G6PD Coimbra and Mahidol variants were reported to be distributed in West Indonesia, whereas Viangchan, Kaiping, and Gaohe G6PD variants were reported in East Indonesia. However, it was noted that G6PD variants, which were found in Eastern Indonesia, were also found in western Indonesia and vice versa.[36] This could be explained by the migration of people who have shared genes with the original population, and thus introducing the G6PD mutation genes. An inconclusive positive correlation was found between ethnicity and the Kaiping variant (P = 0.014), due to the small sample size of the Kaiping variant (only two samples) and Timorese being the most dominant ethnicity in population.

Based on the screening data by the Randox test from the previous study, 96 subjects (25.2%) among the population were G6PD deficient. This number was different from the previous report (92 subjects were G6PD deficient)[24] because four samples with a G6PD level < 6.97 U/gHb were misdiagnosed as normal. The G6PD activity based on the Randox test was associated with Coimbra genotypes (P = 0.011) and hemizygous males with the Vanua Lava variant (P = 0.001). This suggested a correlation between G6PD mutation and enzyme activity. However, normal G6PD activity was also found in the Coimbra variant (8/16 of heterozygous females), Kaiping variant (2/2 of heterozygous females), and Vanua Lava variant (1/2 homozygous female) (Table 4). These results indicated that the G6PD enzyme activity varied in heterozygous females. This phenomenon results from a mosaic effect of expression, as only one X-chromosome in each cell is active. Random inactivation of one or the other X-chromosome (mosaicism) is due to lyonization, which results in heterozygous females having G6PD activities ranging between 0% and 100%. The ratio of the two cell types that make up the mosaic is not the same in all females; thus, they can be expressed both as normal or deficient individuals.[25,37,38] The qualitative tests most commonly used to check for G6PD deficiency in clinical settings are adequate for identifying males with G6PD deficiency, and therefore informing appropriate treatment options. However, these tests do not accurately define G6PD activity in females, especially in heterozygous females. Qualitative testing uses 30% of normal G6PD activity as the cutoff.[18] Because heterozygous females almost exclusively occupy intermediate G6PD enzyme-level phenotypes, mainly ranging from 30% to 80% of normal G6PD activity, qualitative screening tests for G6PD deficiency are insensitive above 30% of normal activity, and this leads to an increased risk of hemolysis by primaquine administration.[39] A cohort study by Chu et al.[40] in areas along the Thai–Myanmar border reported higher daily doses of primaquine as having the potential to cause clinically significant hemolysis in G6PD heterozygous females who are reported as phenotypically normal with point-of-care tests. Thus, molecular analysis may be required in heterozygous females for the disorder.[41]

Glucose 6-phosphate dehydrogenase–deficient subjects were less likely to be infected with malaria than non-deficient individuals. In females, deficient G6PD activity lowered *P. falciparum* infection to 0.0645 times compared with individuals with normal G6PD activity (OR = 0.0645 [95% CI: 0.0085–0.4889] P = 0.008). In males, the deficient activity lowered the infection by *P. vivax* to 0.1690 times compared with individuals with normal G6PD activity (OR = 0.1690 [95% CI: 0.0548–0.5208] P = 0.002). It is postulated that subjects with

TABLE 5
Association between G6PD status and malaria infection in members of the South Central Timor population

		Malaria infection, *n* (%)			
		Plasmodium falciparum	*Plasmodium vivax*	Mixed infection	Non-infection
Female (*n* = 225 [59.1%])	G6PD screening activity (Randox test)*				
	Normal	33 (8.7)	39 (10.2)	16 (4.3)	83 (21.8)
	Deficient	1 (0.2)	12 (3.2)	2 (0.5)	39 (10.2)
		OR = 0.0645			
		(*P* = 0.008)			
	G6PD genotype				
	Coimbra (592C>T)†				
	No mutation	34 (8.9)	48 (12.6)	17 (4.5)	110 (28.9)
	Heterozygous	–	3 (0.8)	1 (0.3)	12 (3.1)
	Kaiping (1388G>A)†				
	No mutation	34 (8.9)	51 (13.4)	18 (4.8)	120 (31.5)
	Heterozygous	–	–	–	2 (0.5)
	Vanua Lava (10884T>C)‡				
	No mutation	33 (8.7)	51 (13.4)	18 (4.7)	121 (31.7)
	Heterozygous	–	–	–	1 (0.3)
	Homozygous	1 (0.3)	–	–	–
Male (*n* = 156 [40.9%])	G6PD screening activity (Randox test)*				
	Normal	18 (4.7)	40 (10.5)	7 (1.9)	49 (12.9)
	Deficient	5 (1.3)	4 (1.0)	4 (1.0)	29 (7.6)
			OR = 0.1690		
			(*P* = 0.002)		
	G6PD genotype				
	Coimbra (592C>T)†				
	No mutation	23 (6.0)	44 (11.5)	11 (2.9)	78 (20.5)
	Kaiping (1388G>A)				
	No mutation	23 (6.0)	44 (11.5)	11 (2.9)	78 (20.5)
	Vanua Lava (10884T>C)‡				
	No mutation	23 (6.0)	43 (11.3)	11 (2.9)	75 (19.6)
	Hemizygous	–	1 (0.3)	–	3 (0.8)

G6PD = glucose 6-phosphate dehydrogenase.
* G6PD screening activity (normal > 6.97 U/gHb and deficient ≤ 6.97 U/gHb).
† All carrier mutation samples were heterozygous female.
‡ Data from the previous study.[24]

G6PD deficiency are protected from malaria infection.[20,42,43] According to Arese et al.,[42] mutations are considered protective if the following three evidence criteria are fulfilled: 1) geographical evidence (their distribution is geographically coincident with the present or historical distribution of malaria plasmodium, and their frequency is correlated with the severity of the disease), 2) epidemiological evidence (the carriers of protective mutations have lower mortality or less severe symptoms), and 3) mechanistic evidence (the mechanism of resistance is clarified by in vitro/in vivo studies). It is still too early to conclude that the results of this study have met the postulate because the hemoglobinopathic protection against malaria has not been substantiated according to those criteria. The possibility of protection against malaria by G6PD mutations deserves further investigation among the SCT population.

The demographic characteristics of the samples revealed that 181 (47.5%) of them were asymptomatic malaria (PCR correction), with a majority of infections by *P. vivax*. A daily dose of 0.25 mg/kg primaquine for 14 days was recommended for the radical treatment of *P. vivax* patients with normal G6PD activity.[13,17] This regimen could reduce the risk of primaquine-induced hemolysis due to unavailable G6PD testing in areas where *P. vivax* is prevalent.[15,16] However, there were some reports that this regimen is less effective at preventing relapse. A study reported in 2017 by Douglas et al.,[44] in a hospital named Rumah Sakit Mitra Masyarakat, Timika, East Indonesia, compared the *P. vivax* treatment with DHP combined with primaquine versus DHP alone. This study followed the primaquine administration policy of 2006, from a lower dose (0.25 mg/kg given daily for 14 days—recommended by Indonesian Ministry of Health) to a higher dose (0.5 mg/kg given daily for 14 days) for radical treatment of vivax. It found that among one-third of the patients who represented at the hospital with *vivax* malaria within 1 year, those treated with primaquine, were only 10% less likely to represent than those who had not received primaquine. Underdosing, as well as incomplete adherence to extended treatment regimens, could have contributed to the poor effectiveness of the drug. Another study of Indonesian soldiers with *P. vivax* acquired in Papua Province demonstrated that a supervised regimen of DHP with a high-dose primaquine regimen (30 mg daily dose or 45 mg daily dose to subjects weighting > 70 kg) had an excellent efficacy of 98% (95% CI: = 91–99%).[45] No studies in Indonesia demonstrated low-dose primaquine (0.25 mg/kg daily for 14 days) efficacy. Considering several reports showing low efficacy of primaquine from malaria sites in Indonesia, the government should reconsider the low-dose primaquine policy for the radical treatment of *vivax* malaria. The WHO recommends 0.5 mg/kg daily for 14 days for patients with normal G6PD activity in areas where frequently relapsing strains of *P. vivax* are prevalent,[14] with additional control for G6PD-deficient patients. Long regimens (14 days) of primaquine administration could play a role in poor adherence to this drug treatment.[46] A recent study by Taylor et al.,[47] in Afghanistan, Ethiopia, Indonesia, and Vietnam, revealed a short-course 7-day-regimen primaquine was well tolerated and non-inferior to 14-day primaquine in patients with normal G6PD. The short-course regimen might improve

adherence and the effectiveness of primaquine for radical cure of *P. vivax* malaria.

In conclusion, there are three G6PD variants (Coimbra, Kaiping, and Vanua Lava) observed in the SCT population. This information has added up on the G6PD genotypes in the population of malaria-endemic area in Indonesia. Molecular analysis of G6PD mutations may be required in vivax patients because of their risk of hemolysis under primaquine treatment. Besides the existing program to prevent artemisinin resistance, Indonesia should evaluate the low-dose primaquine policy for *vivax* radical cure.

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