G6PD deficiency in Myanmar

a brief of available evidence

Background

Several studies have estimated glucose-6-phosphate dehydrogenase (G6PD) deficiency prevalence in Myanmar. However, no nationwide survey has taken place to determine overall G6PD deficiency prevalence. The purpose of this document is to provide background information on methods to measure G6PD and collate all available and accessible data from gray and peer-reviewed literature on G6PD deficiency to provide decision-makers with a comprehensive view of the situation in Myanmar. After collating available data, a draft of this document was shared with members of the extended Technical Strategy Group for Malaria that coordinates malaria partners in Myanmar, and their feedback was incorporated into this document.

What is the G6PD enzyme and what does it do?

The G6PD enzyme plays a critical role in protecting red blood cells from damage and premature destruction from by-products of normal cell processes as well as external challenges. Mutations occur naturally in humans, including on the G6PD gene, and some of these mutations disrupt the normal structure and stability of the enzyme, leading to low levels of G6PD enzyme activity inside the red blood cells, or G6PD deficiency.

Why is G6PD deficiency important for vivax patients?

- World Health Organization (WHO) guidelines state that "the G6PD status of patients should be used to guide administration of primaquine for preventing relapse" of vivax malaria. The text of this recommendation can be found at the end of this document.

- Although mostly asymptomatic, G6PD deficiency can cause acute hemolytic anemia—triggered by infection, ingestion of fava beans or drugs. One such family of drugs are 8-aminoquinolines such as primaquine or tafenoquine.
- To prevent drug-induced hemolytic anemia, it is important to identify a patient's G6PD status before prescribing primaquine or tafenoquine for *P. vivax* malaria.
- Primaquine is the only available antimalarial drug currently recommended globally by WHO for radical cure of *Plasmodium vivax* (*P. vivax*).
- In Myanmar, the treatment for patients infected by *P. vivax* malaria is chloroquine (3 days) and primaquine (14 days).[1] The dosage for primaquine is usually a total dose of 3.5–7 mg/kg over 14 days in G6PD-normal patients or eight weeks of treatment with a weekly dose in G6PD-deficient patients.

How is G6PD classified?

- G6PD status is classified into three categories: normal, intermediate, and deficient.
- G6PD deficiency is an X-linked genetic disorder, and males are typically G6PD deficient or normal. Women can be G6PD deficient, intermediate, or normal.
- G6PD normal is defined as either > 70 percent or > 80 percent of 100 percent G6PD enzyme activity level.
- Those with G6PD activity levels between 30 percent and 70–80 percent are of intermediate status, and typically only females fall into this category.
- Below 30 percent is defined as deficient.[2–4] G6PD enzyme activity level < 10 percent is also sometimes used to define G6PD deficient, typically with males.[2]
- These classifications defined in percentage terms are universal across countries, yet their specific value (units per gram of hemoglobin) will differ by population as seen in the Myanmar-specific evidence table below.
- Universal values for G6PD thresholds are likely to be appropriate in defining G6PD deficiency at the 30 percent level.[5]
- It is expected that for a given G6PD prevalence rate there would be half as many females defined as deficient and twice as many females defined as intermediate.

How is G6PD measured?

- Spectrophotometric assay is considered the "gold standard" for quantitative assessment of enzymatic activity in red blood cells and used to define 100 percent G6PD activity level.[6]

- Quantitative or semi-quantitative (i.e., tests that use a quantitative score to provide a qualitative result) diagnostic tests can determine G6PD normal, intermediate, or deficient status.
- Qualitative G6PD tests can determine if a sample or patient is either above or below the G6PD deficiency threshold of 30 percent activity but cannot typically identify G6PD intermediate status, which is mainly found in females.
- To ensure all patients have access to G6PD testing and uphold principles of gender equity, quantitative or semi-quantitative G6PD diagnostics should be used for patient care.
- Several laboratory-based quantitative assays have been used by researchers to determine G6PD status. These include those manufactured by Trinity Biotech, Randox Laboratories, Pointe Scientific, Sigma-Aldrich, BIOLABO, and Spinreact.[5] Qualitative tests, such as the fluorescent spot test (FST) have also been commonly used in studies, another is the Brewer's Test .
- Point-of-care diagnostics for G6PD deficiency are also now available and have the potential to expand access to well tolerated radical cure and as such enable significant progress toward malaria control and elimination goals. Two quantitative or semi-quantitative G6PD tests are now on the market manufactured by SD BIOSENSOR and Access Bio; the latter also produces a qualitative G6PD rapid diagnostic test. FSTs have also been used for population screening but usually require some basic equipment, electricity, and a functioning cold chain for storage of reagents.[7]

How has G6PD been measured in Myanmar previously?

We identified 17 subnational studies undertaken in nine states in Myanmar (Table 1). Nine measured G6PD prevalence with qualitative or semi-quantitative tests, either the Brewer's test, FST, or formazan test. Five studies measured G6PD quantitatively using either spectrophotometry or point-of-care quantitative tests such as the Trinity Biotech quantitative G6PD or the Access Bio G6PD CareStart™ biosensor.

How prevalent is G6PD deficiency in Myanmar?

- Note: Numbers in parenthesis correspond to study numbers in Table 1.
- G6PD-deficiency prevalence rates varied considerably both between genders and states/ethnic groups (Table 1).
- Overall prevalence of any G6PD deficiency defined as "below normal" (i.e., either intermediate or deficient) ranged from 0 percent (8)–30.5 percent (9). It is expected that the prevalence of G6PD-deficiency in females would be half that observed in men, and that G6PD-intermediate prevalence would be twice as high in females.

- Three studies reported quantitative G6PD deficiency estimates by spectrophotometry for males. Estimates of G6PD deficiency in these studies were 11.7 percent (3), 9.4 percent (5), and 11.1 percent (10).
- Work by Howes, et. al at the Malaria Atlas Project that collates G6PD data with associated location data estimates a G6PD-deficiency prevalence rate of 6.1 percent in Myanmar, or more than 1.5 million males in 2010 and 880,000 females that year.[10]

G6PD deficiency in different ethnic groups

- The most commonly studied group were Burmese (eight studies), Kachin (five studies), and Rakhine (three studies). Chin, Karen, Mon, and Shan were also participants in two studies each, while Kayah and Pa-O populations were identified in one study each. Other ethnicities such as Chinese and Indian were identified in one study.
- The highest prevalence of G6PD deficiency (i.e., either deficient or intermediate) was reported among Kachin populations with a range of 0–30.5 percent G6PD either deficient or intermediate enzyme activity.
- In the one study that used quantitative measures, severe G6PD deficiency (i.e., < 10 percent) was estimated as 2.1 percent in females and 9.4 percent in males.
- The population with the next-highest prevalence of G6PD non-normal enzyme activity were Burmese with a range from 0–21.2 percent. In studies using quantitative measures, G6PD intermediate ranged from 0.9–15.8 percent while G6PD deficient ranged from 0.3–11.7 percent.

What types of G6PD variants are prevalent in Myanmar?

The most commonly reported mutations identified in Myanmar over the last two decades were Mahidol (>= 86 percent of samples), Kaiping (4–18 percent), and Viangchan (6 percent). Others that were identified included Mediterranean (4 percent), Union (2 percent), and Canton (2 percent).

What are the implications of these results on National Malaria Control Program recommendations?

- Studies over time, and in several different populations in Myanmar, have identified populations with moderate to high prevalence of G6PD deficiency up to 30.5 percent prevalence.
- These results strongly support the WHO recommendations to screen G6PD deficiency at the health facility level before the use of primaquine (or any 8-aminoquinoline) prior to radical curative regimen for *P. vivax*.[2]
- Given the variability of G6PD-deficiency prevalence, patient counseling as to the potential side effects of 8-aminoquinolines and a strong pharmacovigilance system are important in Myanmar to ensure patient safety.
- Strategies that increase access to G6PD testing, and strategies to treat *P. vivax* malaria in G6PD-deficient patients, like ensuring adherence to 8-week primaquine, will be important to achieve malaria elimination in Myanmar.

Table 1. Studies measuring G6PD prevalence in Myanmar 1999–2020.[1]

#	Study author (year)	Study location and population	Ethnicity	Gender/ age	N	G6PD deficiency prevalence (%)	Type of test	Definition of deficiency threshold and common variant	Common variant
1	Zeng et al. (2020)[11]	Kachin	Mainly Kachin	All 6–15 yrs	988	16.9	Fluorescent spot test kit (Micky Co. Ltd., Guangzhou, China)	G6PDd =< 40% normal activity	Not reported
2	Yi et al. (2019)[12]	Kachin	Kachin	All	100	Severe—6 Inter—18	Quantitative G6PD assay (Trinity Biotech, St. Louis, MO, USA)	Severe =< 1.0 IU/gHb Intermediate (inter) =< 4.5 IU/gHb	Mahidol 27.2% Kaiping 1.2% Viangchan 0.4% Chinese 0.4%
3	May et al. (2019)[13]	Yangon—children with dengue	Not reported	M	103	Severe—11.7 Inter—1.9	Humalyzer 3000 Spectrophotometric assay and Randox G6PD quantitative *in vitro* test kit	Adjusted male median: 5.7 IU/gHb Severe = 1%–10% AMM Inter = 10%–60% AMM Normal >= 60% AMM	Mahidol 96% Kaiping 4%
				F	93	Severe—2.2 Inter—14			
4	Lee et al. (2018)[14]	Mandalay state—malaria patients	Burmese	M	215	Severe—4.2 Inter—15.8	Multiplex allele-specific PCR kit	Adjusted male median: Not reported Severe = 1%–10% AMM Inter = 10%–60% AMM Normal >= 60% AMM	Mahidol 68%, Kaiping 18% Viangchan 6% Med 4%, Union 2% Canton 2%
				F	37	Severe—8.1 Inter—10.8			
5	Deng et al. (2017)[15]	Kachin state	Kachin	M	53	Severe—9.4 Inter—13.2	Quantitative G6PD assay (Trinity Biotech, St. Louis, MO, USA)	Severe = 0–1.2 IU/gHb Inter = 1.2–< 4.5 IU/gHb Normal >= 4.5 IU/gHb	Mahidol 92%
				F	47	Severe—2.1 Inter—23.4			
6	Han et al. (2017) [16]	Rakhine and Chin	Rakhine and Chin	All	320	Severe—12.5 Inter—14.4	G6PD CareStart Biosensor	G6PD male adjusted mean 6.6 Severe = 1%–10% AMM Inter = 10%–60% AMM	Not reported

[1] Where papers compare different types of G6PD tests, we take results from the test considered as the gold standard for that study.

								Normal = >= 60% AMM	
7	Oo (2017)[17]	Yangon—children 1 month–12 years	Not reported	All	500	Total—9[2]	FST confirmed in G6PDd with spectrophotometry	For G6PDd via FST categories: Severe < 10% normal Inter 10%–< 60% normal Normal >= 60% normal	Not reported
				M	250	Severe—1 Inter—5.6			
				F	250	Severe – 0 Inter—1			
8	Han (2019)[18]	Rakhine and Chin national groups	Rakhine and Chin Chine	All	320	Severe—9.7 Inter—4.7 Norm—85.6	CareStart qualitative RDT (results not reported) and CareStart quantitative biosensor	Adj. male median = 6.4 Severe < 30% normal Inter < 60% normal Normal >= 60% normal	Not reported
9	Li et al. (2015)[9]	Kachin	Kachin	M	671	27.9	Fluorescent spot test	G6PD deficient (< 40% of normal activity) control	Mahidol 90%
				F	1,099	30.5			
10	Oo et al. (2016)[19]	Yangon	Total	M	524	11.1	Spectrophotometric assay	G6PD deficient < 8.28 IU/gHb	Not reported
				F	476	2.1			
			Burmese	M	399	10.1			
				F	363	1.3			
			Karen	M	62	21			
				F	57	0.9			
11	Bancone et al. (2014)[20]	Northwestern Thailand (among Burmese population)	Total	M	478	13.7	FST	G6PD deficient < 11.54 IU/gHb (median activity)	Mahidol 90%
			Burmese	M	354	12.9			
			Karen	M	124	14.1			
12	Oo et al. (2011)[21]	Kayah and Rakhine states	Kayak	M	152	Severe—0 Inter—1.3	Brewer's test and agarose gel electrophoresis (male samples) with formazan test (females)	Inter (male) = 10%–60% enzyme activity Severe (male) < 5% Homo or heterozygous via Formazan stain	Not reported
				F	252	Severe—1.9 Inter—0.41			
			Rakhine	M	151	Severe—0 Inter—3.97			
				F	250	Severe—0 Inter—0.8			

[2] 7.6 when corrected for spectro.

13	Oo et al. (2010)[22]	Chin State	Chin	M	147	Severe—0.68 Inter—4.8	Brewer's test and agarose gel electrophoresis (male samples) with formazan test (females)	Men Severe < 10% normal Inter 10%–< 60% normal Women: Homo- or heterozygous via formazan stain	Not reported
				F	261	Severe—0.38 Inter—2.7			
14	Nuchprayoon et al. (2008)[23]	Samut Sakhon province, Thailand (among Burmese population)	Burmese	M	178	10	G6PD activity assay based on WHO-recommended test (Betke et al. 1967).	G6PD deficiency < 1.5 IU/gHb	Mahidol 86%
			Mon	M	162	12			
15	Than et al. (2005)[24]	Southern Shan state	Pa-O	All	407	16.7	PCR screening using primers for G6PD Mahidol	No thresholds for G6PD activity—presence/no presence of primers only	Mahidol
			Bamar		311	21.2			
			Shan		105	15.2			
			Others		19	21.1			
			Non-ethnic		74	10.9			
16	Jalloh et al. (2004)[25]	Mandalay, Rakhine, and Tanintharyi states	Mainly Burmese (except Sittwe)	M	341	Severe—11.1 Inter—0.9	New formazan substrate (WST-8) and Hirono's method		Not reported
				F	309	Severe—0.3 Inter—7.8			
17	Tantular et al. (1999)[8]	Mon state—village surveys—malaria and non-malaria individuals	Burmese	M	307	7.2	Rapid single-stage screening method	Severely deficient < 10% G6PD activity	Not reported
				F	243	2.9			
			Chinese	M	95	4.2			
				F	96	2.1			
			Indian	M	65	7.7			
				F	81	0			
			Mon	M	44	6.8			
				F	48	0			
			Shan	M	25	8			
				F	34	2.9			
			Kachin	M	9	11.1			
				F	6	0			

Abbreviations: FST, fluorescent spot test; G6PDd, glucose-6-phosphate dehydrogenase deficiency; PCR, polymerase chain reaction; WHO, World Health Organization.

Text from the WHO's Global Malaria Program document titled "Testing for G6PD deficiency for safe use of primaquine in radical cure of *P. vivax* and *P. ovale"* [policy brief].[2]

WHO recommendation:

The G6PD status of patients should be used to guide administration of primaquine for preventing relapse.

- To prevent relapse, treat *P. vivax* or *P. ovale* malaria children and adults (except pregnant women, infants aged < 6 months, women breastfeeding infants aged < 6 months, women breastfeeding older infants unless they are known not to be G6PD deficient and people with G6PD deficiency) with a 14-day course of primaquine at 0.25–0.5 mg base/kg body weight daily in all transmission settings.
- In people with G6PD deficiency, consider preventing relapse by giving primaquine at 0.75 mg base/kg body weight once a week for 8 weeks, with close medical supervision for potential primaquine-induced hemolysis.
- When a patient's G6PD status is unknown and G6PD testing is not available, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of adding primaquine.
- For women who are pregnant or breastfeeding, consider weekly chemoprophylaxis with chloroquine until delivery and breastfeeding are completed; then, on the basis of the woman's G6PD status, treat with primaquine to prevent future relapse.

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