Prevalence of G6PD Deficiency and Distribution of Its Genetic Variants Among Malaria-Suspected Patients Visiting Metehara Health Center, Eastern Ethiopia

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Abstract

Background

Glucose-6-phosphate dehydrogenase (G6PD) is cytosolic enzyme which has a vital role for the integrity and functioning of red blood cells. Lower activity of this enzyme leads to the occurrence of acute hemolytic anemia after exposure to oxidative stressors like primaquine. Primaquine is an important drug for the radical cure of *plasmodium vivax* and blocking transmission of *Plasmodium falciparum* thereby enhancing malaria elimination. However, there is a need to distinguish G6PD deficient individuals and administer the drug with special care due to its hemolytic side effects. The main objective of this study is to determine the prevalence of G6PD deficiency among malaria-suspected individuals.

Methods

A cross-sectional study was conducted from September 2020 to September 2021 in Metehara Health Center, Eastern Ethiopia. A structured questionnaire was used to collect the socio-demographic and clinical information of the study participants. Capillary and venous blood samples were collected based on standard procedures for onsite screening tests, DBS preparation and malaria microscopy. The G6PD enzyme activity was measured by careSTART^{TM} POCT S1. Data was entered and analyzed by using SPSS.

Results

A total of 498 study participants were included in the study, of which 62% (309) were males. The overall prevalence of G6PD deficiency based on the biosensor screening was 3.6% (18/498). Eleven of the G6PD deficient samples had mutations confirmed by gene sequencing analysis. Mutations were detected in G267+119C/T, A376T, and ChrX: 154535443 target genes. A significant association was found between sex and history of previous malaria infection with G6PD deficiency.

Conclusions

The study has shown that the G6PD deficient phenotype exists in Metehara even if the prevalence is not very high. G267+119C/T mutation is the predominant G6PD variant reported. Therefore, malaria patient treatment using primaquine should be closely followed up for any adverse effects.

Background

Glucose-6-phosphate dehydrogenase (G6PD) is a house-keeping enzyme for all cells and a particularly important for the integrity and functioning of red blood cells (RBCs) by catalyzing the production of nicotinamide adenine dinucleotide phosphate (NADPH) that provides the cell reduced form of

glutathione, thus it helps the erythrocytes to survive oxidative stress by reducing hydrogen peroxide and other oxygen radicals (1–4).

Glucose-6-phosphate dehydrogenase deficiency (G6PDd) is an X-linked genetic disorder caused by mutations in the G6PD gene on X-chromosome (Xq28) (2). The X-linkage results in G6PDd of hemizygous males and homozygous females, while heterozygous females acquire two groups of RBCs with either normal or deficient G6PD activities (5, 6). The mutations of G6PD gene results in protein variants with different levels of enzyme activity that are associated with a wide range of biochemical and clinical phenotypes. More than 200 mutations are identified and recognized as a cause of G6PDd which affects over 400 million people worldwide (7–10). According to WHO estimation, 7.5% of the world population are carriers of G6PDd and 2.9% are G6PD deficient (11). Although most variants have only slightly subnormal RBC survival, the Mediterranean variant renders the cells highly susceptible to oxidative stress (12).

Individuals with G6PDd might present a spectrum of disorders including atherosclerosis, cardiovascular disease (13), neonatal jaundice, acute massive hemolysis, renal failure, and chronic hemolytic anemia induced by exposure to certain drugs, infections, fava beans, chemicals, and herbal medicines (10). The 8-aminoquinolines, primaquine (PQ) and tafenoquine (TQ) have similar activities against the pre-erythrocyte stages of *plasmodium species* with in the liver (14, 15). Both PQ and TQ metabolites can oxidize hemoglobin and generate excessive reactive oxygen species which can cause lethal acute hemolytic anemia (AHA) in malaria patients with inherited G6PDd (16). Patients having below 30% of the normal G6PD activities are vulnerable to primaquine induced hemolysis in use of increased dose (30 mg) daily for shorter period (17).

The clinical dilemma created by G6PDd and 8-aminoquinolines demands the health providers either to prescribe anti-relapse therapy with risk of acute hemolytic anemia or withhold therapy with risk of further clinical attacks and onward transmission for patients diagnosed with *P. vivax* infection and unknown G6PD status (18). The prevalence of G6PDd was determined in Ethiopia by different studies such as 8.9% of G6PD A+ nationally (19) and 23.26% in Southern Ethiopia (20). The commonest G6PD mutations detected in the country were G6PDA+, G6PDA-, G267+119C/T and ChrX: 154535443 (21).

In the process of malaria elimination, the role of anti-malarial drugs is vital to block transmission by killing gametocytes and reducing the liver stage hypnozoites of *P. vivax* (17). PQ and TQ are the only licensed drugs to attack the gametocyte stages of all *plasmodium species* and hypnozoites stages of *P. vivax* (21, 22). A WHO clinical trials indicates that 80% of *P. vivax* malaria infections which received non-primaquine drug treatments relapsed within six weeks after completion of treatments (23).

The WHO recommends mass screening of the population in regions where the prevalence of G6PDd is more than 3-5% before the administration of primaquine for elimination purpose (24, 25). Before 1990, primaquine was used in Ethiopia for over a quarter of a century until it was removed from malaria treatment regimen with no documented evidence of adverse effects. Currently it has been re-incorporated into the treatment policy in malaria elimination strategy (22).

Although the Metehara district is known to have increased prevalence of malaria infection, there is limited information about the distribution of G6PDd. Therefore, this study aims to determine the prevalence of G6PDd and genotype of G6PD in Metehara district, Ethiopia. Information of G6PDd in the population will contributes for the timely and successful elimination of malaria from the country.

Methods

Study design and sample collection

This study was conducted in Metehara Health Center, Eastern Ethiopia. Metehara is an administrative town in Fentale Woreda which is located in the Great Rift Valley, about 210 km east of the capital city of the country, Addis Ababa. Its latitude and longitude coordinate is 8°540N/ 39°550E and the average elevation is 947 m (3,107 ft) above sea level. Basaka lake and Awash river are essential water bodies in the study area. The irrigation plantation activity by using the nearby water sources for the industrial farming of sugar cane is suitable for breeding of the Anopheles mosquito. The spread of malaria occurs year-round in this area, with the highest transmission season from September to November and March to May (26). The town has a population of 39,585 (19397 male and 20188 female) and there is one primary hospital and one government health center. Individuals who came to Metehara health center with two or more malaria clinical symptoms were recruited in a cross-sectional study conducted from September 01, 2020 to September 30, 2021. Structured questionnaire was used to capture socio-demographic and clinical information of study participants.

A total of 498 study participants were selected using a quota sampling during the study period. Individuals who came to the health facility for malaria diagnosis with at least two malaria symptoms were asked for consent/assent to participate in the study. The enrollment stopped when the required number of samples attained. Capillary and venous blood collection was performed by trained and experienced professionals (27, 28). DBS samples were collected by placing a drop of whole blood on a circle spot of filter paper (Whatman, Maidstone, UK). After drying, individually packed, labelled, zip-locked in a plastic bag with desiccants, transported to EPHI and stored at -20 ^{o}C freezer until laboratory process (22). Malaria microscopy and malaria diagnosis using carestart^{TM} malaria Pf/Pv (HRP2/pLDH) Ag combo RDT method were carried out at the study sites. All Giemsa stained slides were confirmed for its accuracy by senior expert microscopist.

G6PD phenotype measurement

Measurement of G6PD activity, Hgb, G6PD/Hgb ratio was done using careSTART^{TM} POCT S1(Access Bio, Seoul, Korea) method following the manufacturer's instruction for 498 clinical samples (29). Briefly, for each sample, a G6PD test strip with two drops (20-30 µl) of finger-prick whole blood was added into each G6PD and hemoglobin biosensor sample spots at room temperature. The biosensor simultaneously indicates both the hemoglobin and G6PD results within four minutes and was automatically recorded. Although, the G6PD enzyme activity was primarily expressed in U/dl unit, immediately the machine

changed it to U/g Hg by dividing with the hemoglobin measurement. A blank control was used to calibrate the G6PD biosensor to ensure the reading was zero before the next sample measurement. G6PD enzyme level was normalized by the concentration of hemoglobin (i.e. unit of G6PD enzyme per gram of hemoglobin, U/g Hb). The adjusted male median (AMM) G6PD activity, defined as the median G6PD activity of all male participants after excluding samples with less than 10% of the overall median activity, was calculated. For male study participants, class I is G6PD deficient with < 30% of the AMM activity and class II is normal with > 30% of the AMM activity. For female study participants, G6PD activity < 30%, 30–80%, and > 80% of the AMM activity are considered as G6PD deficient, intermediate, and normal, respectively (21, 30, 31)

G6PD genotyping

Three PCR assays were conducted to determine the G6PD gene mutations of exon 4 - 11. For each PCR assay, water was used in a separate reaction as a negative control. PCR amplification was conducted in a 20 µl reaction mixture containing 2 µl of genomic DNA (~50 ng/µl), 10 µl of 2xMaxima SYBR green PCR master mix (Termo Fisher) and 0.3 µM of each forward and reverse primers. Amplifications were performed with an initial denaturation at 94^{o}C for 3 minutes followed by 38 cycles at 94^{o}C for 30 sec, 55-58^{o}C for 30 sec, and 72^{o}C for 60 sec, with a final 6 min extension at 72^{o}C (21). Amplified PCR products were separated by gel electrophoresis with 1.5% agarose gel at 120V for 2 hours. These PCR products were purified and sequenced on an ABI 3730XI DNA analyzer based on published protocols. Sequences were analyzed on BioEdit. All sequences were aligned to the NCBI reference sequences (NG_009015.2) to verify the specifity of the PCR products. Samples with poor sequencing quality or showed singleton mutations were re-amplified and sequenced. Frequency of all detected mutations were compared between G6PD normal and deficient patient samples (21).

Data analyses

All socio-demographic and clinical data was entered and analyzed using SPSS version-26. Mean, median, and standard deviation were computed for quantitative data. The relative contribution of independent variables for the outcome variable were assessed using logistic regression. A *p-value* of less than 0.05 was considered as significant association between the presence of G6PDd and each contributing factors.

Results

Socio-demographic and clinical characteristics

A total of 498 study participants who have signs and symptoms of malaria disease, were included in the study. The majority of the study participants (62%) were male and (88.4%) were in the age group of ≥15 years. The mean age ± SD of the study participants was 27.1 ± 12.8 years with a range of 4-75 years old. Among malaria suspected patients visiting Metehara Health Center, while 52% (259) were negative for all malaria species, 34.6% (172) were positive for *P. falciparum*, 9.2% (46) were positive for *P. vivax* and 4.2% (21) were positive for both *p. falciparum* and *p. vivax* infections. More than half of the respondents had

no history of previous infection with *plasmodium* species. Most of the study participants developed at least two clinical malaria symptoms dominantly, headache and muscle/joint pain accounts 98.6% (491) and 90.4% (450) respectively (Table 1).

Table 1
Socio-demographic information of the study participants and its association with G6PD status, Metehara Health Center, Eastern Ethiopia, September 2021.

Variables		G6PD status			Total (%)
		Normal n (%)	Intermediate n (%)	Deficient n (%)	
Sex	Male	300(97.1)	0	9(2.9)	309(62.0)
	Female	124 (65.6)	56 (29.6)	9 (4.8)	189 (38.0)
Age group (years)	≤5	3 (100)	0	0	3 (0.6)
	6-14	51 (92.7)	3 (5.5)	1 (1.8)	55 (11.0)
	≥15	370 (84.1)	53 (12.0)	17 (3.9)	440 (88.4)
Residence	Urban	282 (82.9)	46 (13.5)	12 (3.5)	340 (68.3)
	Rural	142 (89.9)	10 (6.3)	6 (3.8)	158 (31.7)
History of malaria infection?	No	261 (84.2)	43 (13.9)	6 (1.9)	310 (62.2)
	Yes	163 (86.7)	13 (6.9)	12 (6.4)	188 (37.8)
Malaria status	Negative	214 (82.6)	39 (15.1)	6 (2.3)	259 (52.0)
	Positive	210 (87.9)	17 (7.1)	12 (5.0)	239 (48.0)
Headache	No	5 (71.4)	1 (14.3)	1 (14.3)	7 (1.4)
	Yes	419 (85.3)	55 (11.2)	17 (3.5)	491 (98.6)
Fatigue	No	123 (87.2)	13 (9.2)	5 (3.5)	141 (28.3)
	Yes	301 (84.3)	43 (12.0)	13 (3.6)	358 (71.7)
Muscle and joint pain	No	40 (83.3)	6 (12.5)	2 (4.2)	48 (9.6)
	Yes	384 (85.3)	50 (11.1)	16 (3.6)	450 (90.4)
Chills	No	216 (85.4)	29 (11.5)	8 (3.2)	253 (50.8)
	Yes	208 (84.9)	27 (11.0)	10 (4.1)	245

					(49.2)
Perspiration (Sweating)	No	224 (83.6)	36 (13.4)	8 (3.0)	268 (53.8)
	Yes	200 (87.0)	20 (8.7)	10 (4.3)	230 (46.2)
Anorexia (Vomiting)	No	354 (85.7)	44 (10.7)	15 (3.6)	413 (82.9)
	Yes	70 (82.4)	12 (14.1)	3 (3.5)	85 (17.1)

Prevalence of G6PD deficiency

The G6PD status determined by calculating the adjusted male median (AMM) G6PD activity (6.9) and the G6PD activities <2.07 u/g Hb, 2.07 – 5.52 u/g Hb, and >5.52 u/g Hb were indicated as G6PD deficient, intermediate and normal, respectively and ranged from 0.2 U/g Hb to 22.3 U/g Hb. The overall prevalence of G6PD deficiency was 3.6% (18/498), i.e., 2.9% (9/309) among males and 4.8% (9/189) among females. The females that showed intermediate (30-80% of AMM) G6PD activity were 11.2% (56/498) (Fig. 1).

Association of G6PD deficiency with socio-demographic and clinical factors

In this study, G6PD deficiency showed differences with various independent factors such as age group, sex and plasmodium infection status. The result showed significant association with sex, where females were three times more affected by G6PD deficiency than males (AOR =3.0, 95% CI; 1.1-8.6, *p-value* = 0.032). Similarly, Individuals with previous history of plasmodium infection were more likely to be G6PD deficient than those who hadn't ever been infected with malaria (AOR = 4.0, 95% CI 1.2-12.7, *p-value* = 0.02) (Table 2).

Table 2
Association between G6PD deficiency with different demographic and clinical factors, September 2021.

Characteristics		G6PDd Status		COR(95% CI)	*p-value*	AOR(95% CI)	*p-value*
		Deficient	Normal				
Sex	Male	9	300	1*		1*	
	Female	9	180	1.7 (0.68-4.3)	0.29	3.0 (1.1-8.6)	0.032
Age group (years)	≥15	16	423	1*		1*	
	6-14	1	54	0.49 (0.06-3.8)	0.49	0.44(0.06-3.5)	0.44
	≤5	1	3	8.8 (0.87-89.4)	0.07	35 (2.6-471)	0.007
Residence	Urban	12	328	1*		1*	
	Rural	6	152	1.08 (0.4-2.93)	0.88	0.9 (0.32-2.6)	0.88
History of malaria infection	No	6	304	1*		1*	
	Yes	12	176	3.5 (1.27-9.4)	0.015	4.0(1.2-12.7)	0.02
Malaria status	Negative	6	253	1*		1*	
	Positive	12	227	2.23 (0.82-6.0)	0.12	2.4 (0.73-7.7)	0.15

G6PD mutations

Molecular analysis was investigated for 17 DBS samples that were G6PD deficient (16) and intermediate (01) based on phenotypic measurement were sequenced for the G6PD exons 4-11. The overall G6PD gene mutations were detected in 64.7% (11/17) of the sequenced samples. The G267+119C/T, G→C was the most common mutation detected in 2.2% (11/498) of the study population, of which nine of them were single base substitution and the two [376, A→T and ChrX: 154535443, G→C] were polymorphic with G267+119C/T, G→C mutation. Previously reported mutations in Ethiopia such as A376G, G202A, G1116A, 485+37 and C563T, were not detected in this study. The predominant single mutation G267+119C/T was detected among 36.4% of male and 63.6% of female phenotypically G6PDd individuals. It was detected in 63.6% and 36.4% of malaria positive and malaria negative phenotypically G6PDd individuals, respectively (Table 3).

Table 3
Distribution of G6PD deficiency genetic variants with respect to sex, age group and malaria status of the study population, Metehara Health Center, Eastern Ethiopia, September 2021.

Characteristics	G6PD genotype					
	G267+119C/T		A376G		ChrX: 154535443	
	Wild type (%)	Mutant G→C (%)	Wild type (%)	Mutant A→T (%)	Wild type (%)	Mutant G→C (%)
Sex						
Male	1 (33.3)	4 (36.4)	4 (40)	0	5 (41.7)	0
Female	2 (66.7)	7 (63.6)	6 (60)	1 (100)	8 (58.3)	1 (100)
Malaria status						
Positive	1 (33.3)	7 (63.6)	7 (70)	0	8 (58.3)	0
Negative	2 (66.7)	4 (36.4)	3 (30)	1 (100)	5 (41.7)	1 (100)
Age group (years)						
≤5	0	1 (9)	1 (10)	0	1 (7.7)	0
5-14	0	1 (9)	1 (10)	0	1 (7.7)	0
≥15	3 (100)	9 (82)	8 (80)	1 (100)	11 (84.6)	1 (100)

Discussion

In this study, phenotypic analysis of G6PD enzyme activity indicated that 3.6% (18/498) of the study participants had <30% enzyme activity of the AMM which is stated as G6PD deficiency and 56 female patients were with intermediate G6PD enzyme activity (30-80%) of AMM assumed to have heterozygous gene mutation.

The G6PDd was relatively higher in females (4.8%) than males (2.8%). This result contradicts the assumption that females are less affected with G6PDd due to genetic preferences of the X-chromosomes (2). The prevalence of G6PDd found in this study was slightly lower than finding from a previous study conducted in seven sites of Ethiopia, which reported 5.4% (10/184) among males and 5.2% (7/136) among females (21). This study also revealed lower G6PDd compared to the study report from other African countries, with a G6PDd of 13% (36/278) (32). The current finding is lower than 7.3% (33/449) reported from Gambella Hospital, Southwest Ethiopia, with 8.6% in males and 6.3% in females (33). A nationally conducted study in Ethiopia discovered 8.9% of G6PDd (19). The G6PDd in this study was higher than Jimma (34) and other community-based study in other parts of Ethiopia that showed relatively low levels of G6PD deficiency (22).

G6PD enzyme activity varies among different nations, regions and even between local ethnic groups. The prevalence of G6PDd was slightly higher among Nuers (14.3%) and Anuak (12.0%) compared to the highlanders with no deficiency in Gambella region (33). Malaria infection might impose G6PD enzyme genetic mutation due to natural selection mechanisms which results in relative incremental of G6PDd in malaria endemic areas compared to non-endemic areas (22). Metehara is one of the seasonal malaria endemic areas in Ethiopia that we observed higher G6PDd prevalence than the community based studies in different settings and lower than similar studies in stable endemic areas like Gambella (33).

Malaria status showed marginal but not statistically significant association with G6PDd, while sex and history of malaria infection were significantly associated with G6PDd status. The prevalence of G6PDd among females was three times higher than in males and it was four times higher among previously malaria infected patients than those never infected individual. In a study report from Gambella Hospital, Ethiopia the prevalence of G6PDd was higher among males than females (33). Likewise, another study conducted in seven study sites of Ethiopia found no significant association of G6PDd with sex and malaria infection (21). Conversely, the incidence of G6PDd among malaria smear positive patients was significantly higher than the malaria negative patients (33).

This variation in associations of various factors with G6PDd prevalence might be due to the difference in ethnic variation of the study participants, endemicity of malaria infection, the sample size and sampling techniques of the studies, the laboratory methods used for the analysis of samples in the study and the time of the study.

Based on molecular sequencing, mutations were detected in G267+119C/T, A376T and ChrX: 154535443 with G267+119C/T being most prevalent. Although previous studies in Ethiopia reported the presence of A376G, G202A, C563T, G1116A and 485+37, these mutations were not identified in this study. Contrary to this study, 13% of the study participants showed G6PDA- (G202A) genotype in Brazaville, Republic of Congo (12), 12.5% (39/311) depicted G6PDA+ (A376G) in Eritrea (35). Several studies in different localities of Ethiopia showed that G6PDA+ (A376G) was the only mutation observed in 8.9% of sampled population (19), and 23.26% (20/86) G6PDA+ mutations were detected in Southwestern Ethiopia (20). The G202A mutation was also detected in 3.5% individuals (22) and in another study, G6PDA+ mutation was detected in 6.1% (21/344) of individuals, G267+119C/T and G1116A mutations found in 1.2% (4) and 1.2% (4) individuals, respectively (21). Whereas no mutation was detected in previous study conducted in Shewa Robit (21) and out of 34 low enzyme activity samples genotyped only one G6PDA+ and one G445A mutations were identified in Oromia region (34).

The A376T mutation represents the exchange of adenine by thyamine, which generates the amino acid replacement of 126 Asn with Tyr. While this mutation is new to Ethiopia, it was previously found in Mexico and termed as San Luis Potosi (36). There were six samples with absence of mutations despite phenotypically showing low enzyme activities. In other study one sample with low G6PD enzyme activity had no G6PD mutation (21). On the other hand, the mutations observed in A376G, G267+119C/T, and G1116A were not associated with low G6PD activity (21). The lack of association of G6PD enzyme

activities with respective genotypes advocates for the need of further verification with large sample size. A codon that has not been sequenced in this study may have contributed to the phenotypic expression of low enzyme activity.

Generally, previous studies that had been conducted in different parts of Ethiopia on the distribution of G6PDd showed that few variants like G6PDA+, at North, south, West, and East of the country were identified (19, 21). G267+119C/T and G1116A were detected in the southern parts of the county (20, 21) and one mutation at position 445G◊A was identified in Jimma (34). This study also identified the pre-existing genotypes as well as the new genotype, A376T mutation. The occurrence of new genetic variants in such a small-scale study suggests the need of a large scale G6PDd epidemiologic study across the country to characterize the full array of G6PD genetic variants in Ethiopia.

Conclusion

The prevalence of G6PD deficiency is low in Metehara district based on phenotypic measurement. Such prevalence was significantly associated with sex and previous malaria infection history of the study participants. G267+119C/T was the predominant genetic variant detected among the participants. Thus, it is recommended to treat patients infected with malaria in Metehara district by primaquine under close supervision and cautious follow up for any hemolytic complications. The presence of the new mutation in Ethiopia give an insights to the presence of various types of G6PD genetic variants in the country. Future study should aim for national-wide epidemiologic study of G6PD deficiency in the country.

Definition

AMM: Adjusted male median, the median of all G6PD enzyme activity of male study participants by excluding values < 10% of the median of the whole participants.

Deficient: The G6PD enzyme activity of both male and female study participants which is less than 30% of AMM.

Intermediate: The G6PD enzyme activity of female study participants which are between 30% and 80% of the AMM.

Non-deficient: The G6PD enzyme activity which are greater than 30% of AMM for male study participants and greater than 80% of AMM for female study participants.

List Of Abbreviations

G6PD
glucose-6-phosphate dehydrogenase
G6PDd
glucose-6-phosphate dehydrogenase deficiency

NADPH
nicotinamide adenine dinucleotide phosphate
RBCs
red blood cells
PQ
primaquine
TQ
tafenoquine
AMM
adjusted male median

Declarations

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Authors’ contributions

TTS, SMF and TK proposed and designed the study; TTS, MTN, AA and DN collected sample and perform preliminary laboratory tests; DK, LW and EL performed molecular laboratory test; TTS, TK, AH, SMF, DN, SGG and EL wrote the paper and analyzed the data. All authors read and agreed to the published version of the manuscript.

Availability of data and materials

The data produced in the study is included in the main manuscript and the rest are available upon reasonable request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Ethical consideration

The study was approved by the institutional review board (IRB) of Addis Ababa University and the IRB of EPHI. The purpose of the study was explained and written informed consent was obtained from each study participants. Participants with malaria infection were treated immediately based on the national malaria treatment guideline free of charge in the study facility. All personal information was kept confidential.

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Consent for publication

Not applicable

References

1. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Molecules Diseases. 2009;42(3):267–78.
2. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. The Lancet. 2008;371(9606):64–74.
3. Bharti RS, Vashisht K, Ahmed N, et al. First report of Glucose-6-phosphate dehydrogenase (G6PD) variants (Mahidol and Acores) from malaria-endemic regions of northeast India and their functional evaluations in silico. *Acta Tropica*. 2019:105252.
4. Peters AL, van Noorden CJ. Single cell cytochemistry illustrated by the demonstration of glucose-6-phosphate dehydrogenase deficiency in erythrocytes. Histochemistry of Single Molecules: *Springer*, 2017. p. 3-13.
5. Valencia SH, Ocampo ID, Arce-Plata MI, Recht J, Arévalo-Herrera M. Glucose-6-phosphate dehydrogenase deficiency prevalence and genetic variants in malaria endemic areas of Colombia. Malaria Journal. 2016;15(1):291.
6. Manganelli G, Masullo U, Passarelli S, Filosa S. Glucose-6-phosphate dehydrogenase deficiency: disadvantages and possible benefits. Cardiovascular Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular Hematological Disorders). 2013;13(1):73–82.
7. Li Q, Yang F, Liu R, et al. Prevalence and molecular characterization of glucose-6-phosphate dehydrogenase deficiency at the China-Myanmar border. PloS ONE. 2015;10(7):e0134593.
8. Ouattara AK, Yameogo P, Diarra B, et al. Molecular heterogeneity of glucose-6-phosphate dehydrogenase deficiency in Burkina Faso: G-6-PD betica selma and santamaria in people with

symptomatic malaria in Ouagadougou. *Mediterranean Journal of Hematology and Infectious Diseases*. 2016;8(1).

9. Francis RO, Jhang JS, Pham HP, et al. Glucose-6-phosphate dehydrogenase deficiency in transfusion medicine: the unknown risks. Vox Sang. 2013;105(4):271–82.
10. Gunawardena S, Kapilananda G, Samarakoon D, et al. Prevalence of G6PD deficiency in selected populations from two previously high malaria endemic areas of Sri Lanka. PloS ONE. 2017;12(2):e0171208.
11. Abolghasemi H, Mehrani H, Amid A. An update on the prevalence of glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice in Tehran neonates. Clin Biochem. 2004;37(3):241–4.
12. Gueye NSG, Peko SM, Nderu D, et al. An update on glucose-6-phosphate dehydrogenase deficiency in children from Brazzaville, Republic of Congo. Malaria Journal. 2019;18(1):57.
13. Thomas JE, Kang S, Wyatt CJ, et al. Glucose-6-Phosphate dehydrogenase deficiency is associated with cardiovascular disease in US Military centers. Tex Heart Inst J. 2018;45(3):144–50.
14. Ahmad SS, Rahi M, Sharma A. Relapses of *Plasmodium vivax* malaria threaten disease elimination: time to deploy tafenoquine in India? BMJ Global Health. 2021;6(2):e004558.
15. Cui Y, Zhang L, Xia Z, Zhou H, Huang F. Epidemiological characterization of imported recurrent *Plasmodium vivax* and *Plasmodium ovale* in China, 2013–2020. Infectious Diseases of Poverty. 2021;10(1):1–10.
16. Baird JK, Surjadjaja C. Consideration of ethics in primaquine therapy against malaria transmission. Trends in Parasitology. 2011;27(1):11–6.
17. Ghimire P, Singh N, Ortega L, et al. Glucose-6-phosphate dehydrogenase deficiency in people living in malaria endemic districts of Nepal. Malaria Journal. 2017;16(1):214.
18. Baird JK. Basic Research of *Plasmodium vivax* Biology Enabling Its Management as a Clinical and Public Health Problem. *Frontiers in Cellular and Infection Microbiology*. 2021;11.
19. Assefa A, Ali A, Deressa W, et al. Glucose-6-phosphate dehydrogenase (G6PD) deficiency in Ethiopia: absence of common African and Mediterranean allelic variants in a nationwide study. Malaria Journal. 2018;17(1):388.
20. Carter TE, Mekonnen SK, Lopez K, et al. Glucose-6-phosphate dehydrogenase deficiency genetic variants in malaria patients in Southwestern Ethiopia. The American Journal of Tropical Medicine Hygiene. 2018;98(1):83–7.
21. Lo E, Zhong D, Raya B, et al. Prevalence and distribution of G6PD deficiency: implication for the use of primaquine in malaria treatment in Ethiopia. Malaria Journal. 2019;18(1):1–10.
22. Shitaye G, Gadisa E, Grignard L, et al. Low and heterogeneous prevalence of glucose-6-phosphate dehydrogenase deficiency in different settings in Ethiopia using phenotyping and genotyping approaches. Malaria Journal. 2018;17(1):281.
23. Organization WH. Guidelines for the treatment of malaria. World Health Organization; 2015.

24. Williams O, Gbadero D, Edowhorhu G, et al. Glucose-6-phosphate dehydrogenase deficiency in Nigerian children. PloS ONE. 2013;8(7):e68800.
25. Thielemans L, Gornsawun G, Hanboonkunupakarn B, et al. Diagnostic performances of the fluorescent spot test for G6PD deficiency in newborns along the Thailand-Myanmar border: a cohort study. *Wellcome Open Research*. 2018;3.
26. Nega D, Assefa A, Mohamed H, et al. Therapeutic efficacy of artemether-lumefantrine (Coartem®) in treating uncomplicated *P. falciparum* malaria in Metehara, Eastern Ethiopia: regulatory clinical study. PLoS ONE. 2016;11(4):e0154618.
27. Organization WH. WHO guidelines on drawing blood: best practices in phlebotomy. World Health Organization; 2010.
28. Organization WH. editor Point-of-care G6PD testing to support safe use of primaquine for the treatment of *vivax* malaria. WHO Evidence Review Group meeting report; 2014.
29. Pengboon P, Thamwarokun A, Changsri K, Kaset C, Chomean S. Evaluation of quantitative biosensor for glucose-6-phosphate dehydrogenase activity detection. PloS ONE. 2019;14(12):e0226927.
30. Ley B, Bancone G, Von Seidlein L, et al. Methods for the field evaluation of quantitative G6PD diagnostics: a review. Malaria Journal. 2017;16(1):1–9.
31. Zobrist S, Brito M, Garbin E, et al. Evaluation of a point-of-care diagnostic to identify glucose-6-phosphate dehydrogenase deficiency in Brazil. PLoS Neglected Tropical Diseases. 2021;15(8):e0009649.
32. Nguetse CN, Meyer CG, Adegnika AA, et al. Glucose-6-phosphate dehydrogenase deficiency and reduced haemoglobin levels in African children with severe malaria. Malaria Journal. 2016;15(1):346.
33. Tsegaye A, Golassa L, Mamo H, Erko B. Glucose-6-phosphate dehydrogenase deficiency among malaria suspects attending Gambella hospital, southwest Ethiopia. Malaria Journal. 2014;13(1):438.
34. Kießling N, Brintrup J, Zeynudin A, et al. Glucose-6-phosphate dehydrogenase activity measured by spectrophotometry and associated genetic variants from the Oromiya zone, Ethiopia. Malaria Journal. 2018;17(1):358.
35. Tseghereda YG, Nganga JK, Kimang'a AN, Mehari TH, Weldemichael YG. Glucose-6-phosphate dehydrogenase deficiency allelic variants and their prevalence in malaria patients in Eritrea. *The Pan African Medical Journal*. 2018;31.
36. Gómez-Manzo S, Marcial-Quino J, Vanoye-Carlo A, et al. Glucose-6-phosphate dehydrogenase: update and analysis of new mutations around the world. Int J Mol Sci. 2016;17(12):2069.

Figures

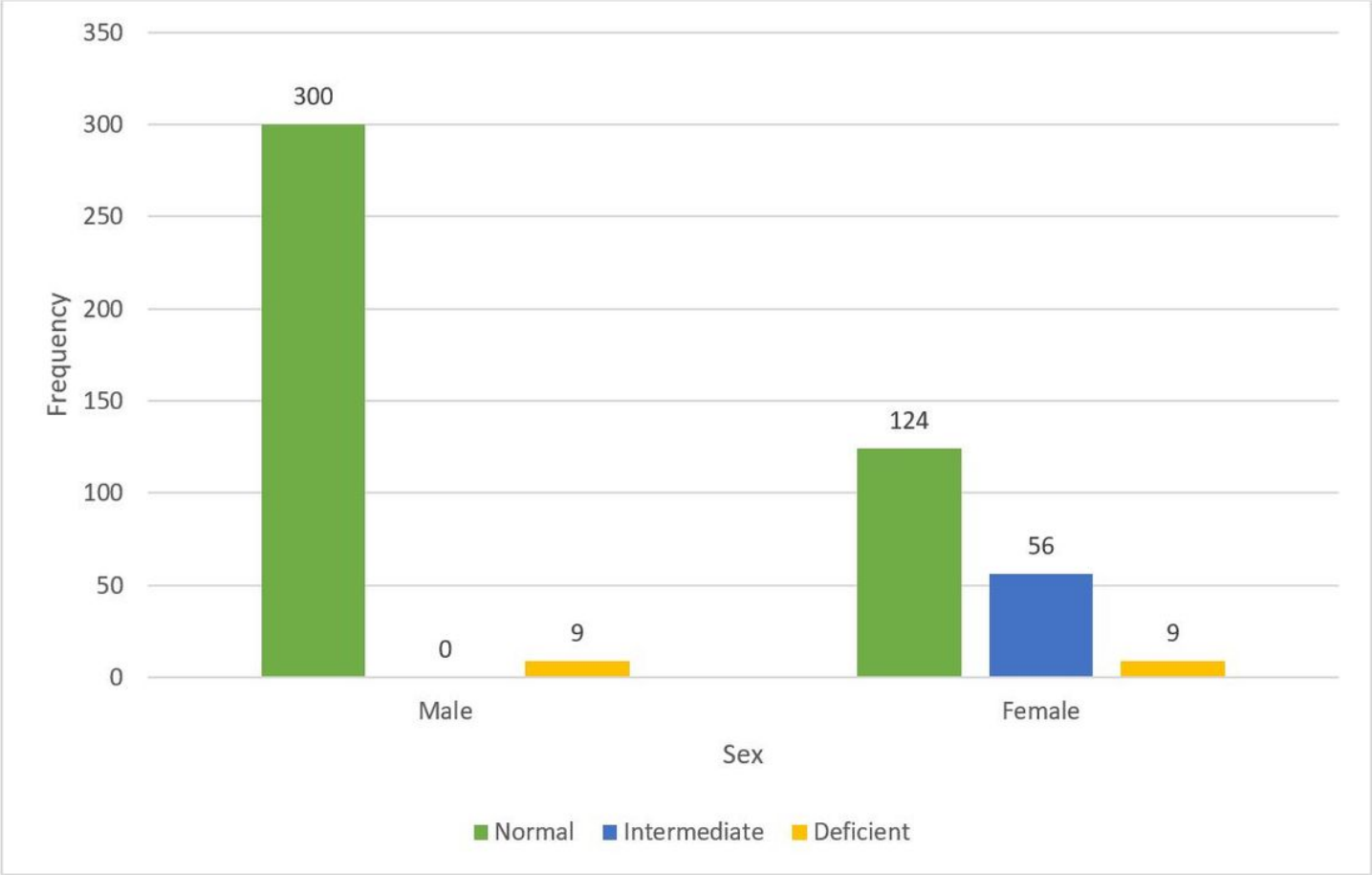

Figure 1

The proportion of patients with G6PD deficient (<2.07 u/g Hb), intermediate (2.07-5.52 u/g Hb) and normal activities (>5.52 u/g Hb) in Metehara Health Center, Eastern Ethiopia, September 2021.

Supplementary Files

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