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Prevalence of Glucose–6–phosphate dehydrogenase deficiency and distribution of its genetic variants among malaria suspected patients in Metehara Health Center, Eastern Ethiopia.

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List of Abbreviations and Acronyms

AAU	Addis Ababa University
AHA	Acute hemolytic anemia
AMM	Adjusted Male Median
Bp	Base pair
CI	Confidence interval
DBS	Dry blood spot
DMIP	Department of Microbiology, Immunology and Parasitology
DNA	Deoxyribose nucleic acid
EPHI	Ethiopian public health institute
G6PD	Glucose-6-phosphate dehydrogenase
G6PDd	Glucose-6-phosphate dehydrogenase deficiency
Hgb	Hemoglobin
IRB	Institutional Review Board
NADPH	Nicotinamide adenine nucleotide phosphate
PCR	Polymerase chain reaction
PQ	Primaquine
RBCs	Red blood cells
SPSS	Statistical package for social science
TQ	Tafenoquine
UNC	University of North Carolina
USA	United States of America
WHO	World health organization

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Abstract

Introduction: Glucose-6-phosphate dehydrogenase is a cytosolic enzyme that has a vital role in the functioning and integrity of red blood cells. Lower activity of this enzyme leads to the occurrence of acute hemolytic anemia after exposure to oxidative stressors like primaquine. Although primaquine is important for the radical cure of *Plasmodium vivax* and blocking the transmission of *Plasmodium falciparum*, thereby enhancing malaria elimination, there is a need to distinguish glucose-6-phosphate dehydrogenase deficient individuals and/or administer the drug with special care due to its hemolytic side effects.

Objective: To determine the prevalence of Glucose-6-Phosphate dehydrogenase deficiency among individuals suspected of malaria during their outpatient visit to Metehara Health Center, Eastern Ethiopia.

Materials and Methods: A cross-sectional study was conducted from 01 September 2020 to 30 September 2021. 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. Data was recorded and analyzed by using SPSS version 23 software.

Result: A total of 498 patients participated in the study; 62% (309) of them were males. According to the results of the biosensor screening test, the overall prevalence of glucose-6-phosphate dehydrogenase deficiency was 3.6% (18/498). There was a significant association between sex and history of previous malaria infection with glucose-6-phosphate dehydrogenase deficiency, P-value 0.032 and 0.02 respectively. Eleven out of the 17 (64.7%) Glucose-6-phosphate dehydrogenase deficient samples by bio-sensor screening test had confirmed mutations using sequencing. The G267+119C/T, A376T, and ChrX: 154535443 mutation types were detected.

Conclusion and Recommendation: Phenotypically, the prevalence of glucose-6-phosphate dehydrogenase deficiency was low. G267+119C/T is the predominant glucose-6-phosphate dehydrogenase variant. In Metehara, Malaria patient management using the drug primaquine under close follow up for developments of any adverse effects is recommended.

Key words: Glucose-6-phosphate dehydrogenase, *Plasmodium vivax, Plasmodium falciparum*, Primaquine, Metehara, Ethiopia.

1. Introduction

1.1. Back-ground

Glucose-6-phosphate dehydrogenase (G6PD) is a housekeeping enzyme for all cells and particularly essential for the integrity and functioning of red blood cells (RBCs) through initiating the production of nicotinamide adenine dinucleotide phosphate (NADPH). G6PD offers cells the reduced form of glutathione that helps the erythrocytes to survive oxidative stress by reducing hydrogen peroxide and other oxygen radicals (Bharti *et al.*, 2019, Cappellini *et al.*, 2008, Nkhoma *et al.*, 2009, Peters *et al.*, 2017). The G6PD gene was cloned in 1986 and comprises a GC-rich promoter, 13 exons, and 12 introns (Manganelli *et al.*, 2013). The monomer of G6PD enzyme has a molecular weight of 59 Killo Dalton and 515 amino acids. The enzyme is active in its dimer or tetramer forms (Cappellini *et al.*, 2008). It is the rate-limiting enzyme of the pentose phosphate pathway (PPP) (Francis *et al.*, 2013, Peters *et al.*, 2017).

Glucose-6-phosphate dehydroginase deficiency (G6PDd) was first described in 1956 as a result of investigations into self-limited hemolysis that happened after administration of antimalarial drug primaquine (PQ) among individuals of African or Mediterranean ethnic origin (Cappellini *et al.*, 2008, Francis *et al.*, 2013, Peters *et al.*, 2017). It is an X-linked genetic disorder caused by mutations in the G6PD gene (Cappellini *et al.*, 2008). Males have only the hemizygous G6PD gene which produces either normal or deficient RBCs, while females have two copies of the G6PD gene that can produce normal, deficient (homozygous), or intermediate (heterozygous) RBCs (Manganelli *et al.*, 2013).

Mutations in the G6PD gene result in protein variants with different levels of enzymatic activity that represent a wide range of biochemical and clinical phenotypes. Currently, the G6PD gene has been identified with more than 400 variants using biochemical diagnosis (Li *et al.*, 2015). Among those 186 mutations affecting the gene coding sequence have been recognized as causes of G6PD deficiency. Of which, at least 35 mutant alleles are polymorphic (Ouattara *et al.*, 2016) while most of these (85%) are single-base substitutions leading to amino acid replacements (Kotepui *et al.*, 2016). Although most variants have only slightly subnormal RBCs survival, the Mediterranean variant renders the cells highly susceptible to oxidative stress (Gueye *et al.*,

2019). In Africa, G6PDd is typical of the A- variant whereas G6PD*B and G6PD*A+ are wild types and non-deficient variants respectively (Shitaye *et al.*, 2018).

Individuals with G6PDd may experience a range of diseases, including atherosclerosis, cardiovascular disease (Thomas *et al.*, 2018), acute massive hemolysis, neonatal jaundice, renal failure, and chronic hemolysis induced by exposure to a small number of drugs, infections, faba beans, chemicals, and herbs (Gunawardena *et al.*, 2017). G6PD-deficient RBCs are susceptible to destruction by oxidative stress, indicated as acute hemolytic anemia (AHA) (Kotepui *et al.*, 2016, Pal *et al.*, 2021, Peters *et al.*, 2017).

The 8-amonoquinolines; PQ and tafenoquine (TQ) have similar drug activity against the pre-erythrocyte stages of *Plasmodium species* within the liver (Ahmad *et al.*, 2021, Cui *et al.*, 2021). Both PQ and TQ metabolites can oxidize hemoglobin and generate excessive oxygen radicals, which may lead to fatal AHA in malaria patients with inherited G6PD deficiency (Baird *et al.*, 2011). For this reason, WHO recommends G6PD test before using PQ or TQ to eradicate the malaria parasite (Organization, 2015).

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 (Baird, 2021). The widespread use of 8-aminoquinolines (PQ and TQ) has been restricted due to its potential to encourage hemolytic anemia in G6PD-deficient individuals. Hemolytic anemia in individuals with G6PD deficiency ranges from mild to life-threatening diseases (Assefa *et al.*, 2018). Because of the considerable progress made in malaria control, Ethiopia's vision is to be malaria-free by 2030 (Assefa *et al.*, 2018, Shitaye *et al.*, 2018). Determining the G6PDd prevalence and variants in the study area can help the respective malaria-endemic districts health offices to follow new approaches to implement and scale up the use of PQ and TQ for both *P. falciparum* transmission reduction and *P. vivax* radical cure, which is very essential in achieving their vision during malaria elimination program.

1.2. Statement of the problem

Glucose-6-phosphate dehydroginase deficiency is one of the commonest inherited enzymopathy, affecting over 400 million people globally (Amoah *et al.*, 2021, Francis *et al.*, 2013, Gunawardena *et al.*, 2017). According to WHO estimation, 7.5% of the world population are carriers of G6PDd and 2.9% are G6PD deficient (Abolghasemi *et al.*, 2004). The highest prevalence of G6PDd is in tropical Africa, the Middle East, tropical and subtropical Asia, Papua New Guinea, and various Mediterranean regions (Cappellini *et al.*, 2008).

Glucose-6-phosphate dehydroginase deficient variants may remain asymptomatic in the absence of inducing agents. Clinical manifestations caused by G6PD variants may vary with gradients of clinical conditions from asymptomatic to severe (Lee *et al.*, 2018). Patients having below 30% of the normal G6PD activities are vulnerable to PQ induced hemolysis in use of increased dose (30 mg) daily for a shorter period (Ghimire *et al.*, 2017, Taylor *et al.*, 2021) and G6PDd is also a challenge for use of TQ as prophylaxis or treatment of hypnozoite stage of *P. vivax* malaria (Markus, 2021). But the problem can be minimized by adjusting the dose and length of PQ administration time to 15 mg daily for 14 days (Howes *et al.*, 2013).

According to the 2011 national malaria indicator survey result, Ethiopia was found to have 8.9% prevalence of G6PD* A (A376G) variant mutation. The prevalence of the G6PD * A (A376G) mutation varied slightly across the country. The leading prevalence was observed in the peoples of southern nations and nationalities (12.2%) and in the Tigray regions (12%) (Assefa *et al.*, 2018). This mutation was also detected in 20/86 (23.26%) of patients in Southern Ethiopia (Carter *et al.*, 2018).

According to the 2020 WHO report, malaria remains a major health problem worldwide. About 229 million infections and 409,000 deaths occur in 2019 world wide with 94% of morbidity and mortality rates identified in Africa (Organization, 2020). In Ethiopia, 2.8 million malaria cases and 5000 deaths have been reported every year, and *P. vivax* and *P. falciparum* are species recognized to coexist (Carter *et al.*, 2018). *P. vivax* is highly prevalent and contributes to 44% of malaria cases in the country (Carter *et al.*, 2018) and 10% of the global *vivax* malaria cases (Shitaye *et al.*, 2018). The 2019/2020 EFY report revealed that a total of 2544 malaria cases had been diagnosed in all government and private health service organizations in Metehara town, of

which *P.falcparum* and *P.vivax* contributed 1809(71%) and 735(29%) of the total cases, respectively *(information taken from Metehara town administration health office)*.

Among malaria elimination efforts, antimalarial drugs are critical for malaria elimination, with a focus on the role of drugs in blocking the transmission of malaria by destroying gametocytes and reducing the pool of *P. vivax* and *Plasmodium ovale* hypnozoites in the liver stage (Ghimire *et al.*, 2017). Primaquine and TQ are the only licensed drugs to attack the gametocyte stage of all *Plasmodium species* during infection and hypnozoite stages of *P. vivax* during its dormant stage to ensure the radical cure of infected person (Lo *et al.*, 2019, Shitaye *et al.*, 2018).

A WHO clinical trial in Indonesian soldiers showed that about 80% of *P. vivax* malaria infections who received non-PQ drug treatments relapsed within six weeks after completion of treatments (Organization, 2015). A cohort study in Papua New Guinea showed that approximately 80% of *P. vivax* episodes occurred by activating the incubation period of the liver rather than new infections, while another similar study in Western Thailand estimated that this proportion was more than 90%, and every person infected with *P. vivax* carries about five dormant bodies in the liver (Baird, 2021). Although PQ is useful in malaria control and elimination programs, it also has the potential to harm individuals who are G6PD deficient (Carter *et al.*, 2018).

WHO recommends mass screening of the population in regions where the prevalence of G6PDd is more than 3-5% before the administration of PQ for elimination purposes (Thielemans *et al.*, 2018, Williams *et al.*, 2013). Before 1990, PQ was used in Ethiopia for over a quarter of a century until it was removed from the malaria treatment regimen with no documented evidence of adverse effects. Currently, it is re-incorporated into the treatment policy in malaria elimination strategy (Shitaye *et al.*, 2018).

Although the area is known to have an increased prevalence of malaria infection, there is limited information about the distribution of G6PDd in the study area. The status of G6PDd in the population needs to be determined, which helps to eliminate malaria from the country in a timely and successful manner. Therefore, this study aimed to determine the prevalence of G6PDd and distribution of its genetic variants in the Metehara district.

1.3. Significance of the study

- The use of PQ is indicated for *P. falciparum* transmission interruption and *P. vivax* radical cure. But it is contraindicated for G6PD deficient individuals because of its predisposition to cause AHA. The study will initiate quantitative measurement and provide information on the prevalence of G6PDd in the study area.
- There are variants of G6PD gene mutations with gradients of enzymatic activities which lead to its classification. The severity of clinical outcomes of G6PDd depends on the type of G6PD variants. So this study informs stakeholders the most common variants distributed in the study area.
- The information about prevalence, genotypic variation, and enzymatic activities of G6PD in the study population will contribute to guide malaria treatment strategy using the drug PQ to enhance malaria elimination. This in turn initiates the use of PQ and eases malaria elimination processes.

2. Literature Review

The global prevalence of G6PDd is abundant in geographical areas historically exposed to endemic *Plasmodium* infections. The prevalence of G6PD deficient alleles was estimated to be 8% across all malaria-endemic countries of the World (Howes *et al.*, 2013). According to a meta-analysis of random effect estimates, the overall global prevalence of G6PDd was 7.1% of which about 470 million people were estimated to be affected and it was highest in sub-Saharan Africa compared to estimates from the Middle East, being the second highest. During this review, the male average prevalence estimate of G6PDd for Sub-Saharan Africa was 8.5% (Nkhoma *et al.*, 2009) (Fig. 1).

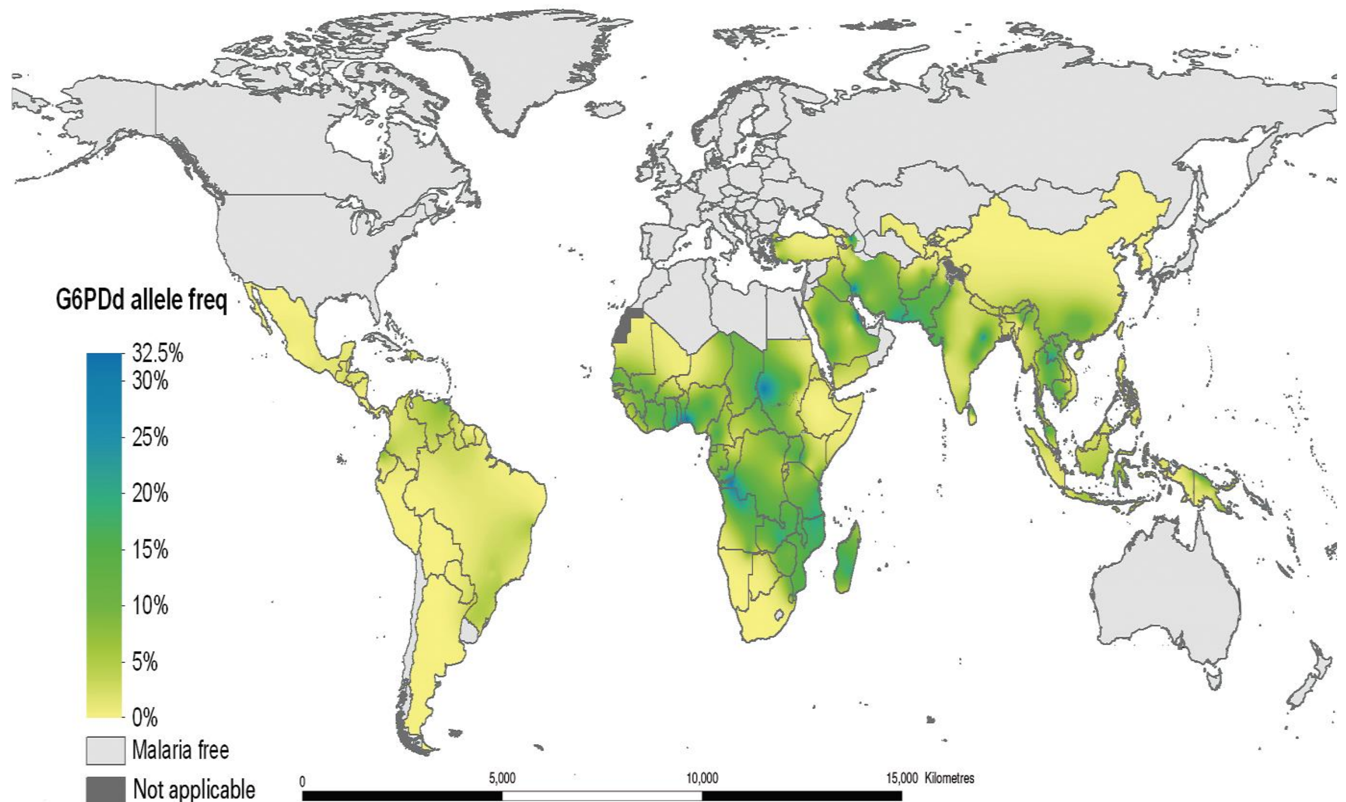

Figure 1. Estimated distribution of G6PD deficiency across the world.

(Source: WHO 2018; G6PD User Guide, Estimated G6PD deficiency allele's frequency in malaria-endemic countries) (Organization, 2018)

The study conducted in Tehran on "prevalence of Glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice" from April to December 1999 showed that G6PDd with a

prevalence of 2.1% (42/2000) contributes to neonatal jaundice among newborns. Of these newborns 36 (85.7%) were boys and 6 (14.3%) were girls. Out of 1623 newborns screened for the development of jaundice, 16% (265/1588) newborns were with normal G6PD compared to 51% (18/35) G6PD-deficient patients who had neonatal jaundice (Abolghasemi *et al.*, 2004).

A cross-sectional study conducted in Sistan and Balouchestan province, Southeastern Iran, between September and December 2010 showed that mild and severe G6PD deficiency was detected in 14.8% and 12.2% of subjects, respectively. Mild G6PD deficiency was identified in male (10.8%) and female (18.9%) subjects. However, a greater proportion of males (15.3%) were found to have severe deficiency than females (9.1%) (Tabatabaei *et al.*, 2015).

A cross sectional study conducted in two hospitals from previously malaria-endemic areas of Sri Lanka indicates that the prevalence of G6PDd in the sample was found to be 13.95% and 7.97% in the Teaching Hospitals of Anuradhapura and Kurunegala, respectively. The prevalence of G6PDd was meaningfully larger in Anuradhapura than in Kurunegala. The prevalence of enzyme deficiency among females was comparable to that of males (Gunawardena *et al.*, 2017).

A retrospective study done on internationally adopted children revealed the G6PDd prevalence of 2.1% had at least one abnormal test. Of all G6PD deficient individuals, 83% with low G6PD activity were identified from children of 12 different countries. Democratic Republic of Congo, Haiti, and Vietnam were countries with the highest prevalence of G6PD deficiency. Thirteen children had been identified for G6PD genetic mutation. Four had G6PD-Mediterranean, of whom two were hemizygous males and two were heterozygous females. The children came from Russia, Bulgaria, Kazakhstan, and other Eastern European countries. Five children had G6PD A−, four were hemizygous males and one was a homozygous female which was from the Democratic Republic of Congo (Spring *et al.*, 2018).

A study conducted in National Blood Transfusion Center in Baghdad city, Iraq from 28th April to 26th August 2008 showed that among 1810 individuals enrolled in the study, 109 (6.0%) were found to be G6PD deficient. Further molecular studies were done for adequately extracted DNA samples of 101 deficient cases. Among 101 G6PD deficient males, 75 (74.3%) were the Mediterranean variant (563 C$\rightarrow$T), 5 (5.0%) were G6PD Chatham (1003 G$\rightarrow$A), and 2 (2.0%)

were G6PD A- (202 G→A). None of the remaining 19 cases showed the G6PD Aures (143 T→C) when tested for this variant (Al-Musawi *et al.*, 2012).

According to the study conducted on the prevalence of G6PDd in malaria infected patients, from January 2013 to April 2015, two hundred forty-five individuals were identified with a malaria infection at the Phop Phra Hospital, Thailand. 217 cases (88.6 %) were infected with *P. vivax*, while the remaining 28 cases (11.4 %) were infected with *P. falciparum*. Among the G6PDd patients, two (11.8 %) were diseased with *P. falciparum* and 15 (88.2 %) were infected with *P. vivax*. The percentage of individuals with *P. falciparum* infection and G6PDd is the same as that of individuals with non-G6PDd (~11%). This clearly demonstrates that the presence of G6PDd does not protect against the *P. falciparum* infection (Kotepui *et al.*, 2016).

According to a study conducted in Upper Myanmar, South Korea, the overall frequency of G6PDd is about 19.9% (50/252), of which 20.0% (43/215) are male and 18.9% (7/37) are females. Among malaria patients with G6PD variants, 50.0% (25/50), 34.0% (17/50), and 16.0% (8/50) were infected with *P. falciparum*, *P. vivax*, and co-infected with both *P. falciparum* and *P. vivax*, respectively. Six different G6PD allelic variants were identified by multiplex allele-specific PCR sequencing analysis. The most common G6PD mutation identified was the Mahidol variant (68%, 34/50). Other least detected variants are Kaiping (18%, 9/50), Viangchan (6%, 3/50), Mediterranean (4%, 2/50), Union (2%, 1/50) and Canton (2%, 1/ 50) (Lee *et al.*, 2018).

In a study conducted in malaria-endemic areas of Colombia among 426 volunteers, 28 individuals (6.56 %) indicated either severe or intermediate G6PD deficiency. Of these 28 patients, six (1.41 %; 2 females and 4 males) were with severe G6PDd, where 22 (5.15 %) individuals presented with intermediate deficiency (Valencia *et al.*, 2016).

A study conducted in a settlement for internally displaced people and five villages near Laiza city identified 523 (29.6%) individuals as G6PD deficient. There sex differences in the prevalence of G6PDd was insignificant; with 27.9% (187/671) males and 30.5% (336/1099) females being G6PD deficient. One hundred ninety eight unrelated G6PD deficient subjects were selected for genotyping. Of which, 156 have mutations in at least one of the 7 remaining loci.

Five mutations related with recognized G6PD deficiency were detected in 43.9% (87/198) of the study population. The Mahidol 487G>A type was the most predominant mutation occurring in 89.7% (78/87) of the subjects and the Kaiping 1388G>A mutation was found in 8.0% (7/87) individuals. Chinese 4392G >T, Canton 1376 G>T, and Viangchan 871G>A were less identified variants (Li *et al.*, 2015).

In 2003, a cross-sectional study conducted in Muheza, North-eastern Tanzania shown that the prevalence of malaria was 17.2% and 39.6% in the highlands and lowlands of the study area respectively. The prevalence of G6PDd was also significantly higher in lowlands than highlands (G6PDd= 11.32% vs. 4.43%) of the study site (Segeja *et al.*, 2008). The prevalence of G6PDd among neonates in Egypt was 4.3% (119/2782), of which 91 were males and 28 were females. The prevalence of G6PDd was higher in males (6.2%, 91/1453) than in females (2.1%, 28/1329) (El-Menshay *et al.*, 2009).

In a cross-sectional study done at Mbingo Baptist Hospital in Cameroon between January 2016 to August 2017, among 1512 blood donors screened for G6PDd in those over 18 years, 7.1% were G6PD-deficient (Lauden *et al.*, 2019). In another cross-sectional study in Eritrea between August and October 2016 among malaria patients, the total prevalence of G6PDd was 12.5% (39/311). All were G6PD A variant, 7.4% were A hemizygous males (type A), 4.8% were A heterozygous females (type AB), and 0.3% were an A homozygous female (type AA). The proportion of the G6PD A variant was higher in males (13.1%) than in females (11.9%) (Tseghereda *et al.*, 2018).

In Ethiopia, molecular genotyping analysis from stored DBS samples collected during the 2011 malaria indicator survey showed that the national prevalence of G6PD deficiency was 8.9%. G6PD*A (A376G) was the only mutation identified. A minor regional difference was observed in the prevalence of G6PD * A (A376G) mutations. Prevalence was higher in Southern Nations, Nationalities, and people (12.2%) and in the Tigray region (12.0%) than in Dire Dawa and Harari, where G6PD mutations were not detected. The prevalence of G6PD * A (A376G) was 9.2% in areas below 2000 m and 7.9% in areas 2000-2500 m above sea level (Assefa *et al.*, 2018).

Another study conducted among malaria suspects attending Gambella Hospital in November-December 2013 showed a total G6PDd prevalence of 7.3%. The occurrence of G6PDd varied between ethnic groups. The proportion of the G6PD-deficient was higher among Nuers (14.3%) and Anuak (12%) than the ‘highlanders’ (all non-deficient). In both cases, the differences were statistically significant ($p < 0.0001$). The incidence of malaria infections among G6PD-deficient individuals was significantly higher than among normal individuals for the enzyme (Tsegaye *et al.*, 2014).

Another molecular study in Jimma town identified G6PDd individuals among malaria patients. The common G6PD variant A376G mutation was detected in 23.26% (20/86) of patients. Nineteen of them were females heterozygous for A376G, and one was male hemizygous for A376G (Carter *et al.*, 2018). According to community and school-based cross-sectional studies conducted in different malaria-endemic settings in Ethiopia. The overall G6PDd was 1.4%, with considerable variation between study sites. Sixteen were from Gambela Regional State (6.6% of participants from Abobo and 1.5% of participants from Lare Woredas) (Shitaye *et al.*, 2018). Another study conducted at Jimma University between March and June 2014, among 206 participants from the Oromia region in Ethiopia, found no G6PD deficiency (Kießling *et al.*, 2018).

3. Objective of the study

3.1. General objective

- To determine the prevalence of G6PD deficiency and distribution of its genetic variants among malaria suspected individuals visiting Metehara Health Center, Eastern Ethiopia, 2021.

3.2. Specific objectives

- To determine the prevalence of G6PD deficiency among malaria suspected individuals visiting Metehara Health Center, Eastern Ethiopia.
- To assess possible factors associated with the G6PD deficiency among malaria suspected individuals visiting Metehara Health Center, Eastern Ethiopia.
- To identify genotypic variants of G6PD gene mutations among malaria suspected individuals visiting Metehara Health Center, Eastern Ethiopia.

4.Materials and Methods

4.1. Study area

The study was conducted in Metehara Health Center, Metehara town, East Shewa zone, Oromia regional state of 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 location is indicated as 8°540N/ 39°550E and has an elevation of 947 m (3,107 ft) above sea level. Lake Basaka and Awash and Germama rivers 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 from September to November and March to May (Nega *et al.*, 2016). The town has a population of 39,585 (19397 male and 20188 female) and there is one Primary Hospital and one government health center in the town.

4.2. Study design and period

Individuals who came to Metehara Health center with two or more malaria clinical symptoms were recruited in a cross-sectional study that has been conducted from 01 September 2020 to 30 September 2021. Socio-demographic and clinical information of study participants were collected by a structured questionnaire.

4.3. Target Population

All individuals who have been living in the Metehara district were the target population of the study.

4.4. Source Population

Individuals who came to Metehara Health Center during the study period.

4.5. Study Population

All self-presented individuals to Metehara Health Center who were suspected to be malaria cases during the study period.

4.6. Inclusion and Exclusion criteria

4.6.1. Inclusion criteria

- Living or having lived in the study area for at least 6 months.
- Individuals suspected to have malaria infection.

4.6.2. Exclusion criteria

- Severely ill patients
- Children if blood sample collection is difficult.

4.7. Study variables

4.7.1. Independent variables

- Age
- Sex
- History of malaria infection
- Malaria status

4.7.2. Dependent variables

- Prevalence of G6PD deficiency
- Genetic variants of G6PD mutations

4.8. Sample size determination

The minimum sample size was calculated using the single population proportion formula by taking the expected G6PDd prevalence of 0.5, desired precision of 5% and 95% confidence interval (CI).

Using a single population proportion formula

$$n = z^2 \times p\,(1-p)/d^2$$

Where n = sample size,

Z = Z statistic for a level of confidence (95% level of confidence; z=1.96),

P = expected prevalence or proportion (P= 0.5)

d = precision (in proportion of precision 5%, d = 0.05).

⇨ $n = (1.96)^2 * 0.5*(1-0.5)/0.05^2$

- ⇨ n = 384
- ⇨ 10% non-respondents were added = 38
- ⇨ So based on this calculation the minimum sample size of the study was 384 + 38 = 422, but due to the presence of adequate resources the sample size increased to 498 and collected during the study period.

4.8. Sampling methods and procedures

The study participants were selected using a quota system during the study period. Any person who came to the health facility for malaria diagnosis with at least two malaria symptoms was assessed for inclusion/exclusion criteria and asked for his/her consent/assent for participation in the study. The selection stopped when the required number of samples was attained.

4.9. Laboratory analysis

Laboratory investigations were conducted at different settings based on the requirements of each method. 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, which had 100% sensitivity and 96% specificity measured against standard quantitative G6PD enzyme activity test method (Pengboon *et al.*, 2019). Initial malaria microscopy and diagnosis using CaresStart^{TM} malaria pf/pv (HRP2/pLDH) Ag combo RDT method were done at the study sites. All Giemsa stained slides read at the health center were collected and sent to Adama malaria center for rechecking by senior expert microscopists. Further molecular characterization of G6PD genetic variants was performed at the University of North Carolina at Charlotte, USA after proper collection, processing, storage and transport of DBS samples (*see annexes XIII and XIV for detailed procedures of each method*).

4.9.1. Sample collection and transportation

Capillary and venous blood collection performed by trained and experienced professionals who are thoroughly familiar with the collection procedure, use of universal precautions against contamination of finger prick and needle puncture site during collection and understand the quality of specimen for providing reliable test results (Organization, 2010, Organization, 2014).

Dry Blood Spot samples were collected by placing a drop of whole blood on a circle spot of filter paper (Whatman, Maidstone, UK) and dried with air. After drying, the specimens were

individually packed, labeled, zip-locked in a plastic bag with desiccants, transported to EPHI, and stored at -20 ^{o}C freezer until laboratory process (Shitaye *et al.*, 2018).

The memorandum of understanding and material transfer agreement documents were signed by EPHI and UNC for molecular characterization of G6PD using DBS samples at UNC, USA. Then, the DBS samples of all G6PD deficient and 10% of G6PD normal patients were sent to UNC at Charlotte, USA through Fedex postal agent after fulfilling all the international biological sample transportation rules and requirements.

4.9.2. G6PD phenotype measurement

G6PD enzyme levels were measured by CareSTART™ POCT S1 (Access Bio, Seoul, Korea) for 498 clinical samples following the manufacturer's instruction. In short, a G6PD test strip with two drops (20-30 µl) of finger-prick whole blood was added into each G6PD and hemoglobin biosensor sample spot at room temperature for each sample. The biosensor simultaneously indicates both the hemoglobin and G6PD results within four minutes 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. The G6PD biosensor was calibrated using a blank control 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/gHb). According to the WHO classification, individuals with normal and defective G6PD are divided into two male categories and three female categories. The adjusted male median (AMM) G6PD activity was determined by calculating the median G6PD activity of all male participants after samples with less than 10% of the overall median activity were excluded. For males, with < 30% of the AMM activity defined as class I (G6PD deficient) and with > 30 % of the AMM activity class II (normal). For females, G6PD activity < 30%, 30–80%, and > 80% of the AMM activity are classified as G6PD deficient, intermediate, and normal respectively (Ley *et al.*, 2017, Lo *et al.*, 2019, Zobrist *et al.*, 2021).

4.9.3. G6PD Genotyping

Molecular G6PD genotype analysis was carried out at UNC in Charlotte, USA. Three PCR assays were done 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°c for 3 minutes followed by 38 cycles at 94°c for 30 sec, 55-58°c for 30 sec, and 72°c for 60 sec, with a final 6 min extension at 72°c (Lo *et al.*, 2019). 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. NCBI reference sequences (NG_009015.2) were used to verify the specificity of the PCR products. Samples with poor sequencing quality/showed singleton mutations were re-amplified and sequenced. The frequency of all detected mutations was compared between G6PD normal and deficient patient samples (Lo *et al.*, 2019).

4.10. Data analysis

Data were entered and analyzed using SPSS version-23 software. Data were used to describe different characteristics of study subjects by associating dependent and independent variables. The mean, median, standard deviation, table and graphs were used to summarize the data. The relative contribution of independent variables for the outcome variables was assessed using logistic regression. A P-value of less than 0.05 was considered as a significant association between the presence of G6PDd and each contributing factor.

4.11. Quality assurance

All laboratory activities were carried out based on the standard operating procedures of EPHI. Pre-analytical, analytical and post-analytical phases were followed for their quality. Internal and external quality control materials were done for each test method as per the manufacturer's instructions. Any uncertain results were repeated conditionally and reviewed by other laboratory technical experts. All quality assurance activities and results of tests were recorded and documented strictly and reviewed daily for their completeness. The overall activities of the study were followed and monitored regularly by the assigned supervisor.

4.12. Result dissemination

The result which will be produced from this study will be submitted to the Department of Microbiology, Immunology and Parasitology, Addis Ababa University. The result will also be disseminated to EPHI, Fentale woreda health office, Metehara town administration health office,

and other concerned bodies related to this public health issue program according to the university and other Ethical Regulations. Finally, it will be presented at different scientific conferences and the full manuscript will be published in International or national peer-reviewed journals.

4.13. Ethical consideration

The study was approved by the institutional review board (IRB) of the Department of Microbiology, Immunology and Parasitology, Addis Ababa University and the IRB of EPHI. The purpose of the study was explained and written informed consent was obtained from each study participant for individuals aged under 18 years consent form was signed by their parents/guardians and assent was signed by the age groups 11-18 years. A blood sample was collected from each study participant with minimal risk by experienced phlebotomists and was examined by the respective methods for G6PDd and malaria infection. Participants with malaria infection were treated immediately based on the national malaria treatment guideline in the study facility. All personal information was kept confidential.

4.14. Operational 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 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 is greater than 30% of AMM for male study participants and greater than 80% of AMM for female study participants.

5. Result

5.1. Socio-demographic and Clinical Characteristics

A total of 498 study participants who showed signs and symptoms of malaria were included in the study. Most of the study participants (62%) were males 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 and falls in a range of 4-75 years old. Most of the participants were urban residents (68.3%) (*Table 1*).

Table 1. Sociodemographic information of the study participants, Metehara Health Center, Eastern Ethiopia, September 2020 to 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)
	≤5	3 (100)	0	0	3 (0.6)
Age group	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)
Address	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)

All study participants were interviewed for the presence of chronic infections, 99.2% of them responded as being free of any chronic diseases. Among malaria suspected study participants, 52% (259) were malaria negative, 34.6% (172) were *P. falciparum*, 9.2% (46) were *P. vivax* and 4.2% (21) were mixed (Pf + Pv). More than half of the study participants did not have a history of previous *Plasmodium* infection. Headache (98.6%) and muscle/joint pain (90.4%) were the dominant symptoms observed on malaria suspected patients (Table 2).

Table 2. Clinical characteristics of the study participants, Metehara Health Center, Eastern Ethiopia, September 2020 to September 2021.

Variables		G6PD status			Total (%)
		Normal n (%)	Intermediate n (%)	Deficient n (%)	
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)
Did you have complications due to malaria	No	411 (84.7)	56 (11.5)	18 (3.7)	485 (97.4)
	Yes	13 (100)	0	0	13 (2.6)

5.2. Prevalence of G6PD deficiency

G6PD enzyme activity ranged from 0.2 U/g Hb to 22.3 U/g Hb. Based on the WHO guidelines, the G6PD status was determined by calculating the adjusted male median (AMM) G6PD activity after excluding samples with values less than 10% of the overall median activity. For males, G6PD deficient category is <30% of the AMM activity (<2.07) and normal >30% of the AMM

activity (>2.07). For females, G6PD activity <30% (<2.07) is deficient, 30–80% (2.07-5.52) intermediate, and >80% (>5.52) of the AMM activity were considered as G6PD normal.

The overall prevalence of G6PD deficiency was 3.6% (18/498), of which 2.9% (9/309) were males and 4.8% (9/189) were females. Females with intermediate (30-80% of AMM) G6PD activity held 11.2% (56/498) proportion (Fig. 2).

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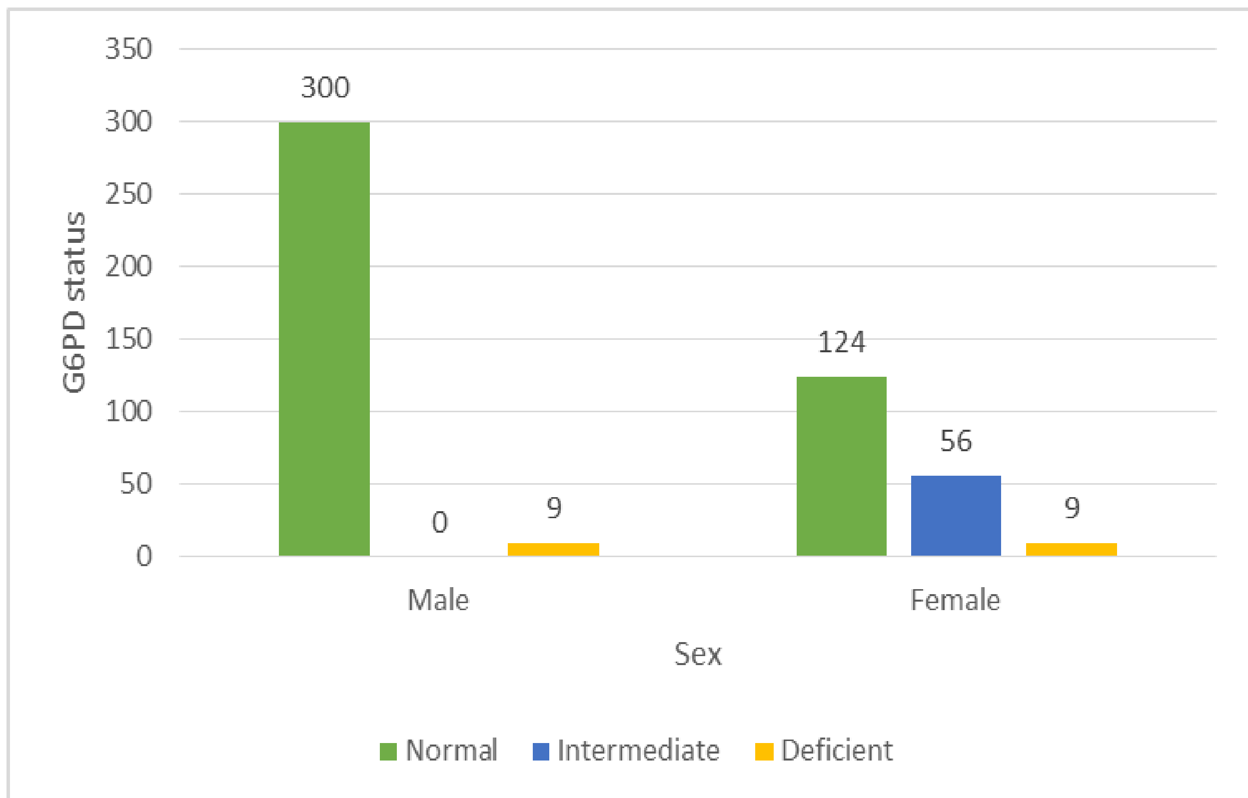


Figure 2. Bar graph indicates the proportion of patients with G6PD deficient, intermediate, and normal activities in Metehara Health Center, Eastern Ethiopia.

5.3. Association of G6PD deficiency and independent factors

In this study, G6PD deficiency showed differences among various independent factors such as age, sex, and *Plasmodium* infection status. The prevalence of G6PD deficiency in male and female study participants was 2.9% (9/309) and 4.8% (9/189), respectively. The result showed a significant association between sex and G6PD activity where females were three times more affected than males (AOR =3.0, 95% CI: 1.1-8.6, p-value = 0.032). Similarly, the previous history of plasmodium infection showed a significant association with G6PD deficiency. Among

all study participants, 37.8% (188/498) had previous malaria infection, of which 12/188 participants were G6PD deficient while only 6/310 of the non-infected were G6PD deficient. Those who had previous malaria infection were four times 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 3).

The G6PD deficiency among malaria infected and non-infected individuals were 5% (12/239) and 2.3% (6/259) respectively. *Plasmodium* infected patients had higher G6PD deficiency compared to the non-infected patients, but it was not significant, (P-value = 0.15) (Table 3).

Table 3. Association between G6PD deficiency and different independent factors in Metehara Health Center, Ethiopia.

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

5.4. Glucose-6-phosphate dehydrogenase deficiency genetic mutations

Since G6PDd is a genetic-based enzymopathy, over 200 mutations of G6PD were recognized worldwide. In this study, molecular analysis was investigated for a total of 17 DBS samples which were phenotypically G6PD deficient (16) and intermediate (01) by careSTART^{TM} POCT S1 rapid diagnostic method at the study site. The DBS samples were sequenced for the G6PD exons 4-11 of the X chromosome. The mean G6PD/Hgb ratio of the samples selected for

molecular gene sequencing was 1.22 U/g Hb with minimum and maximum values of 0.2 U/g Hb and 2.10 U/g Hb respectively. Phenotypically deficient enzyme activity values ranged from 0.2 U/g Hb to 1.9 U/g Hb while the intermediate enzyme activity value was 2.10 U/g Hb. The overall G6PD gene mutations were detected in 11/17 (64.7%) of the sequenced samples. The G267+119C/T, G→C was the most common mutation detected in 11 (2.2%) of the study population, of which nine were single base substitution and two 376, A→T and ChrX: 154535443, G→C were polymorphic with G267+119C/T, G→C mutation. These two mutations, A376T and ChrX: 154535443 were detected in each of the two study subjects. None of the previously reported mutations in Ethiopia such as A376G, G202A, G1116A, 485+37, and C563T were detected in this study (Table 4).

Table 4. Molecular analysis results of phenotypically G6PD deficient/intermediate samples tested for selected target gene mutations at Metehara, Ethiopia.

Genotypic variants	Phenotypic characteristics		Total
	Intermediate	Deficient	
No mutation	0	06	06
A376G	0	0	0
G267+119C/T & A376T	01	0	01
G267+119C/T & chrX: 154535443	0	01	01
G267+119C/T	0	09	09
C563T	0	0	0
485+37	0	0	0
G1116A	0	0	0
Total	01	16	17

Among selected samples for sequencing, ten were females (phenotypically; 9 deficient and one intermediate) and seven were males (phenotypically; all are deficient). Regarding malaria status; eight were infected with *P. falciparum*, two were infected with *P. vivax* and the rest seven were malaria negative. The single mutation G267+119C/T was predominant and detected in 4 (36.4%) males and 7 (63.6%) female individuals. This mutation was detected in 7 (63.6%) malaria positives and 4 (36.4%) malaria negative individuals (Table 5). There was no significant

difference seen among malaria status, sex, and age group of individuals for the G6PD mutation results.

Table 5. Distribution of G6PD deficiency genetic variants concerning the sex, age group, and malaria status of the study population, Metehara Health Center, Ethiopia.

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 (66.6)	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						
≤5yrs	0	1 (9)	1 (10)	0	1 (7.7)	0
5-14yrs	0	1 (9)	1 (10)	0	1 (7.7)	0
≥15yrs	3 (100)	9 (82)	8 (80)	1 (100)	11 (84.6)	1 (100)

Although all sequenced samples had low enzyme activities, 6 out of 17 (35.3%) samples revealed no genetic mutation. The mean G6PD/Hb ratio of the non-mutated genotype was 1.26 U/g Hb with a minimum and maximum values of 0.60 U/g Hb and 1.9 U/g Hb, respectively. Whereas the mean G6PD/Hb ratio of the mutated genotype was 1.30 U/g Hb with a minimum and maximum values of 0.20 U/g Hb and 2.10 U/g Hb, respectively (Fig. 3).

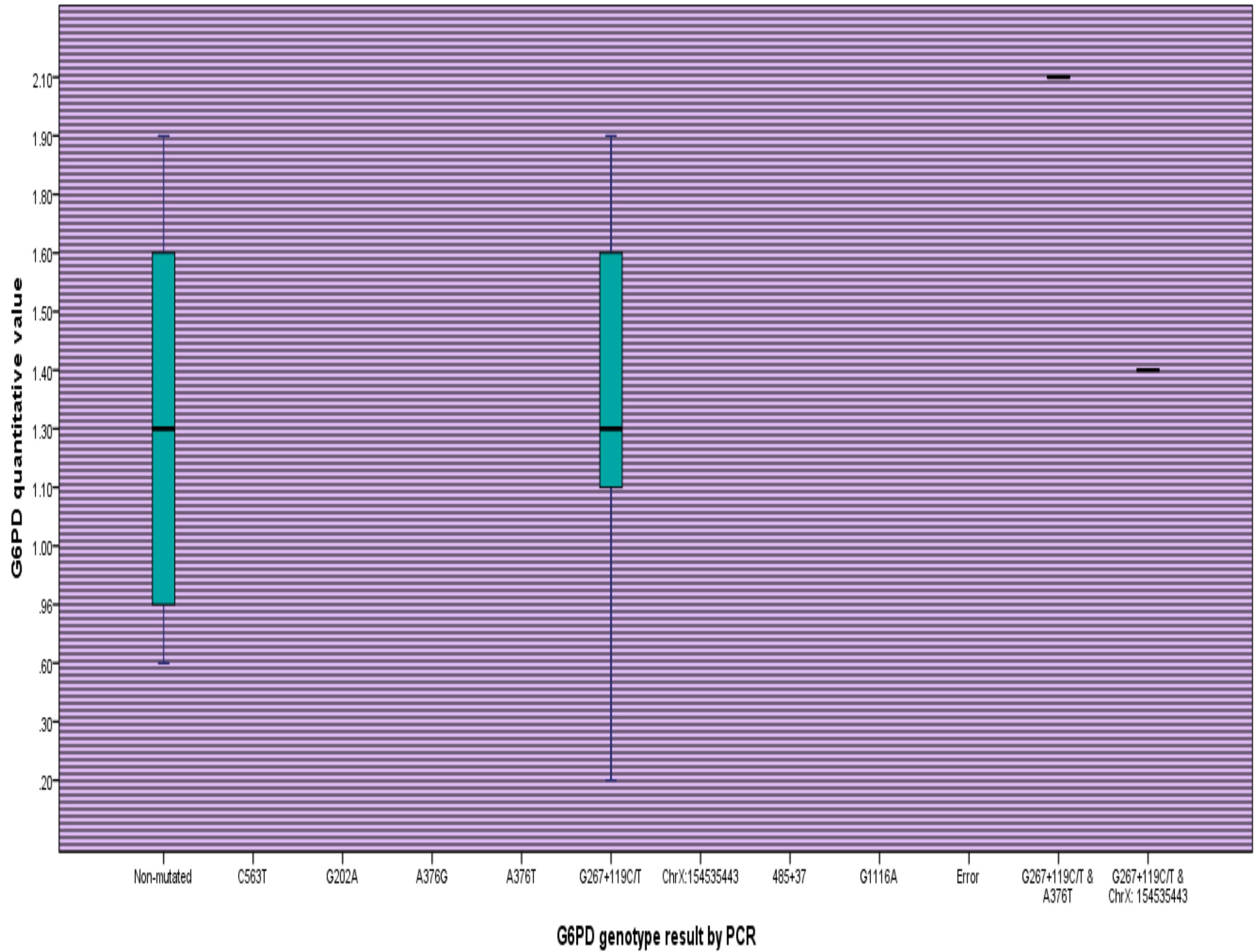


Figure 3. Boxplot chart shows the distribution of G6PD deficient genetic variants with respects to its enzyme activity values in Metehara Health Center, Ethiopia.

6. Discussion

In this study, the adjusted male median (AMM) G6PD activity of 6.9 U/g Hb was considered as a reference for calculating the G6PD status as deficient (<30% of AMM or <2.07 U/g Hb), intermediate for females (30% - 80% of AMM or 2.07 – 5.52 U/g Hb) and normal (>30% of AMM for males or >80% of AMM for females). The 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 enzyme deficiency and 56 female patients were with intermediate G6PD enzyme activity (30%-80%) of AMM assumed to have a 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 (Cappellini *et al.*, 2008) and slightly lower than the finding from a study conducted in seven sites of Ethiopia; between 2013 and 2016 which reported the overall G6PDd as 4.3% (17/400) among which 5.4% (10/184) in males and 5.2% (7/136) were in females (Lo *et al.*, 2019). The variation of G6PD deficiency prevalence between male and female might be impacted by the type of mutation that leads to the lower enzyme activity may not have equal sex preference.

This study also revealed lower G6PD deficiency compared to the study report from other African countries with G6PD deficiency of 13% (36/278) (Nguetse *et al.*, 2016). The current finding is lower than 7.3% (33/449) G6PDd reported from Gambella Hospital, Southwest Ethiopia, with 8.6% in males and 6.3% in females (Tsegaye *et al.*, 2014), and another nationally conducted study in Ethiopia which revealed 8.9% of G6PD*A (A376G) genotype as the only mutation (Assefa *et al.*, 2018).

The G6PDd in this study was higher than the result of a study from Jimma University, Ethiopia which revealed no G6PD enzyme activity <30% of AMM or no G6PD deficient participants identified in the study at all (Kießling *et al.*, 2018) and other community-based studies in Ethiopia which showed 1.4% (22/1609) G6PD deficiency (Shitaye *et al.*, 2018).

The difference observed in the prevalence of G6PDd between our study and other similar studies might be due to ethnic variation of the study participants in the study area and other sites such as Gambella, since G6PDd is a genetic problem, malaria endemicity and medical history of the

study participants, for which this result revealed higher G6PDd result than the community-based studies and lower G6PDd result than the studies in the areas with higher malaria prevalence.

Glucose-6-phosphate dehydrogenase enzyme activity varies among different nations, regions, and even between local ethnic groups. The prevalence of G6PDd was higher among Nuers (14.3%) and Anuak (12.0%) compared to the nil deficient highlanders in the Gambella region (Tsegaye *et al.*, 2014). There is an ideal explanation that malaria infection might impose G6PD enzyme genetic mutation due to natural selection mechanisms which result in relative incremental of G6PDd in malaria-endemic areas compared to non-endemic areas (García-Magallanes *et al.*, 2014). Our result supports such idea that Metehara is one of the seasonal malaria-endemic areas in Ethiopia; so that G6PDd was higher than the community-based studies in different settings and lower than similar studies in stable endemic areas like Gambella (Tsegaye *et al.*, 2014).

In the current study, we have investigated the association between the G6PD deficiency and potential independent factors such as; sex, age group, history of malaria infection, and malaria status. Malaria status did not shows statistically significant association, but sex and history of malaria infection had shown significant association with G6PDd status. The prevalence of G6PDd among females was three times higher than in males (AOR = 3.0, 95% CI; 1.1-8.6, p-value = 0.032) and it was four times higher among previously malaria-infected patients than those never infected individuals (AOR= 4.0, 95% CI; 12-12.7, p-value = 0.02). This showed variation from the study report in Gambella Hospital, Ethiopia that although it was not statistically significant, the prevalence of G6PDd was higher among males than females (Tsegaye *et al.*, 2014). Likewise, another study conducted in seven study sites of Ethiopia found no significant association between G6PDd and related factors: sex, malaria infection, and age of study participants (Lo *et al.*, 2019). Conversely, the incidence of G6PDd among malaria smear-positive patients was significantly higher than the malaria-negative patients (Tsegaye *et al.*, 2014).

This variation in associations of independent factors with the prevalence of G6PDd might be due to medical status of the study participants. This study includes solely the study participants with malaria sign and symptoms. The presence of these clinical symptoms is suggestive for many

clinical circumstances and stimulates various immune responses that can make the difference in the prevalence of G6PDd or the clinical onset may be influenced by the G6PD deficiency.

The genetic sequencing of 17 DBS samples for three G6PD gene regions and then, detection of target gene mutations was carried out. For all molecular sequencing, the mutations of target genes including A376G, G202A, C563T, G1116A, G267+119C/T, Chrx: 154535443, and 485+37 were considered. A total of 11/17 (64.7%) G6PD mutations were detected and three G6PD variants such as G267+119C/T, A376T, and ChrX: 154535443 were identified. The predominant mutation was G267+119C/T, detected among eleven of the samples and two more mutations; A376T and ChrX: 154535443 were also identified on individuals who had G267+119C/T mutations. Although it was noticed that most of the 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 (Gueye *et al.*, 2019), 12.5% (39/311) depicted G6PDA+ (A376G) in Eritrea (Tseghereda *et al.*, 2018), and studies in different areas of Ethiopia showed that 8.9% G6PDA+ (A376G) was the only mutation (Assefa *et al.*, 2018), 23.26% (20/86) G6PDA+ mutations were detected in Southwestern Ethiopia (Carter *et al.*, 2018). The G202A mutation was also detected in 3.5% of individuals (Shitaye *et al.*, 2018) and 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 (Lo *et al.*, 2019). Where no mutation was detected in a previous study conducted in Shewa robit (Lo *et al.*, 2019) and out of 34 low enzyme activity samples genotyped only one G6PDA+ and one G445A mutations were identified in Oromia region (Kießling *et al.*, 2018).

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 (Gómez-Manzo *et al.*, 2016). There were six samples with the absence of mutations that phenotypically showed low enzyme activities. Previously, in a study by Lo E. et.al, one sample with as low G6PD enzyme activity as 1.69 U/g Hb had no G6PD mutation. On the other hand, the mutations observed in A376G, G267+119C/T, and G1116A were not associated with low G6PD activity (Lo *et al.*, 2019). The lack of association of G6PD enzyme activities with respective genotypes advocates for the need

for further verification with a large sample size. Another codon that has not been sequenced in this study may contribute to the phenotypic expression of 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 (Assefa *et al.*, 2018, Lo *et al.*, 2019). G267+119C/T and G1116A were detected in the southern parts of the county (Carter *et al.*, 2018, Lo *et al.*, 2019) and one mutation at position 445G→A was identified in Jimma (Kießling *et al.*, 2018). 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 for a large-scale G6PDd epidemiologic study across the country to characterize the full array of G6PD genetic variants in Ethiopia.

7. Conclusion

The prevalence of G6PD deficiency was low based on its biochemical activity measurement. It was significantly associated with the sex and previous malaria infection history of the study participants. Genotypic analysis revealed that G267+119C/T was the predominant genetic variant detected in the study.

8. Recommendation

The low prevalence of G6PDd in this study directs the health care providers, health system program directors, and other stakeholders to deploy PQ treatments for its critical benefit for the radical cure of *P. vivax* and transmission blockage of *P. falciparum* infection. So, it is recommended to treat patients infected with malaria in Metehara district by PQ under close supervision and cautiously follow up by trained health professionals for its hemolytic complications.

The presence of the new mutations additional to the pre-existing ones in Ethiopia gives insights into the presence of various types of G6PD genetic variants in the country. Based on this evidence, the Ministry of Health, Health system managers, and researchers should aim for a larger national epidemiologic study of G6PD deficiency.

The G6PD enzyme activity is measured quantitatively which gives gradients of enzyme activities, and its' low level expose patients to hemolytic anemia in the presence of potential oxidative stressors. Researchers to design cohort studies that can evaluate the effects of PQ treatment on different background levels of enzyme deficiencies.

9. Limitation

The spectrophotometer method was not used for quantitative measurement of the G6PD enzyme activity that would be important to confirm the careSTAR^{TM} POCT S1 results and compare the agreement with PCR results.

The molecular sequencing of the G6PD enzyme was not done for all phenotypically normal and most intermediate samples that would be very essential to investigate the association of the phenotypic results with its respective genotypic results.

The study was done at a single study area, Metehara which did not consider various ethnicity of the study populations that might inflict the difference in the prevalence of G6PD deficiency and multiplicities of genetic mutations.

10. References

Abolghasemi H, Mehrani H &Amid A 2004. An update on the prevalence of glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice in Tehran neonates. *Clinical Biochemistry,* 37(3)**,** 241-4.

Ahmad SS, Rahi M &Sharma A 2021. Relapses of *Plasmodium vivax* malaria threaten disease elimination: time to deploy tafenoquine in India? *BMJ Global Health,* 6(2)**,** e004558.

Al-Musawi BM, Al-Allawi N, Abdul-Majeed BA, Eissa AA, Jubrael JM &Hamamy H 2012. Molecular characterization of glucose-6-phosphate dehydrogenase deficient variants in Baghdad city-Iraq. *BMC Blood Disorders,* 12(1)**,** 4.

Amoah LE, Asare KK, Dickson D, Abankwa J, Busayo A, Bredu D*, et al.* 2021. Genotypic glucose-6-phosphate dehydrogenase (G6PD) deficiency protects against *Plasmodium falciparum* infection in individuals living in Ghana. *PloS ONE,* 16(9)**,** e0257562.

Assefa A, Ali A, Deressa W, Tsegaye W, Abebe G, Sime H*, et al.* 2018. Glucose-6-phosphate dehydrogenase (G6PD) deficiency in Ethiopia: absence of common African and Mediterranean allelic variants in a nationwide study. *Malaria Journal,* 17(1)**,** 388.

Baird JK 2021. Basic Research of *Plasmodium vivax* Biology Enabling Its Management as a Clinical and Public Health Problem. *Frontiers in Cellular and Infection Microbiology,* 11.

Baird JK &Surjadjaja C 2011. Consideration of ethics in primaquine therapy against malaria transmission. *Trends in Parasitology,* 27(1)**,** 11-6.

Bharti RS, Vashisht K, Ahmed N, Nayak A, Pande V &Mishra N 2019. 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***,** 105252.

Cappellini MD &Fiorelli G 2008. Glucose-6-phosphate dehydrogenase deficiency. *The Lancet,* 371(9606)**,** 64-74.

Carter TE, Mekonnen SK, Lopez K, Bonnell V, Damodaran L, Aseffa A*, et al.* 2018. Glucose-6-phosphate dehydrogenase deficiency genetic variants in malaria patients in Southwestern Ethiopia. *The American Journal of Tropical Medicine and Hygiene,* 98(1)**,** 83-7.

Cui Y, Zhang L, Xia Z, Zhou H &Huang F 2021. Epidemiological characterization of imported recurrent *Plasmodium vivax* and *Plasmodium ovale* in China, 2013–2020. *Infectious Diseases of Poverty,* 10(1)**,** 1-10.

El-Menshay AA, Khalifa NM, Awad SA, Fathy MA &Amer AK 2009. Prevalence of glucose-6-phosphate dehydrogenase deficiency in jaundiced neonates in Egypt. *Australian Journal of Basic and Applied Sciences,* 3(3)**,** 2016-23.

Francis RO, Jhang JS, Pham HP, Hod EA, Zimring JC &Spitalnik SL 2013. Glucose-6-phosphate dehydrogenase deficiency in transfusion medicine: the unknown risks. *Vox Sanguinis,* 105(4)**,** 271-82.

García-Magallanes N, Luque-Ortega F, Aguilar-Medina E, Ramos-Payán R, Galaviz-Hernandez C, Romero-Quintana J*, et al.* 2014. Glucose-6-phosphate dehydrogenase deficiency in northern Mexico and description of a novel mutation. *Journal of Genetics,* 93(2)**,** 325-30.

Ghimire P, Singh N, Ortega L, Rijal KR, Adhikari B, Thakur GD*, et al.* 2017. Glucose-6-phosphate dehydrogenase deficiency in people living in malaria endemic districts of Nepal. *Malaria Journal,* 16(1)**,** 214.

Gómez-Manzo S, Marcial-Quino J, Vanoye-Carlo A, Serrano-Posada H, Ortega-Cuellar D, González-Valdez A*, et al.* 2016. Glucose-6-phosphate dehydrogenase: update and analysis of new mutations around the world. *International Journal of Molecular Sciences,* 17(12)**,** 2069.

Gueye NSG, Peko SM, Nderu D, Koukouikila-Koussounda F, Vouvoungui C, Kobawila SC*, et al.* 2019. An update on glucose-6-phosphate dehydrogenase deficiency in children from Brazzaville, Republic of Congo. *Malaria Journal,* 18(1)**,** 57.

Gunawardena S, Kapilananda G, Samarakoon D, Maddevithana S, Wijesundera S, Goonaratne LV*, et al.* 2017. Prevalence of G6PD deficiency in selected populations from two previously high malaria endemic areas of Sri Lanka. *PloS ONE,* 12(2)**,** e0171208.

Howes RE, Battle KE, Satyagraha AW, Baird JK &Hay SI 2013. G6PD deficiency: global distribution, genetic variants and primaquine therapy. *Advances in parasitology. Elsevier*.

Kießling N, Brintrup J, Zeynudin A, Abduselam N, Götz S, Mack M*, et al.* 2018. Glucose-6-phosphate dehydrogenase activity measured by spectrophotometry and associated genetic variants from the Oromiya zone, Ethiopia. *Malaria Journal,* 17(1)**,** 358.

Kotepui M, Uthaisar K, PhunPhuech B &Phiwklam N 2016. Prevalence and hematological indicators of G6PD deficiency in malaria-infected patients. *Infectious Diseases of Poverty,* 5(1)**,** 36.

Lauden SM, Chongwain S, Achidi A, Helm E, Cusick SE, Krug A*, et al.* 2019. Prevalence of glucose-6-phosphate dehydrogenase deficiency in Cameroonian blood donors. *BMC Research Notes,* 12(1)**,** 195.

Lee J, Im Kim T, Kang J-M, Jun H, Lê HG, Thái TL*, et al.* 2018. Prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency among malaria patients in Upper Myanmar. *BMC Infectious Diseases,* 18(1)**,** 131.

Ley B, Bancone G, Von Seidlein L, Thriemer K, Richards JS, Domingo GJ*, et al.* 2017. Methods for the field evaluation of quantitative G6PD diagnostics: a review. *Malaria Journal,* 16(1)**,** 1-9.

Li Q, Yang F, Liu R, Luo L, Yang Y, Zhang L*, et al.* 2015. Prevalence and molecular characterization of glucose-6-phosphate dehydrogenase deficiency at the China-Myanmar border. *PloS ONE,* 10(7)**,** e0134593.

Lo E, Zhong D, Raya B, Pestana K, Koepfli C, Lee M-C*, et al.* 2019. Prevalence and distribution of G6PD deficiency: implication for the use of primaquine in malaria treatment in Ethiopia. *Malaria Journal,* 18(1)**,** 1-10.

Manganelli G, Masullo U, Passarelli S &Filosa S 2013. Glucose-6-phosphate dehydrogenase deficiency: disadvantages and possible benefits. *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders),* 13(1)**,** 73-82.

Markus MB 2021. Safety and Efficacy of Tafenoquine for *Plasmodium vivax* Malaria Prophylaxis and Radical Cure: Overview and Perspectives. *Therapeutics and Clinical Risk Management,* 17**,** 989.

Nega D, Assefa A, Mohamed H, Solomon H, Woyessa A, Assefa Y*, et al.* 2016. Therapeutic efficacy of artemether-lumefantrine (Coartem®) in treating uncomplicated *P. falciparum* malaria in Metehara, Eastern Ethiopia: regulatory clinical study. *PLoS ONE,* 11(4)**,** e0154618.

Nguetse CN, Meyer CG, Adegnika AA, Agbenyega T, Ogutu BR, Kremsner PG*, et al.* 2016. Glucose-6-phosphate dehydrogenase deficiency and reduced haemoglobin levels in African children with severe malaria. *Malaria Journal,* 15(1)**,** 346.

Nkhoma ET, Poole C, Vannappagari V, Hall SA &Beutler E 2009. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells, Molecules, and Diseases,* 42(3)**,** 267-78.

Organization WH 2010. *WHO guidelines on drawing blood: best practices in phlebotomy*, *World Health Organization*.

Organization WH 2014. How to safely collect blood samples from persons suspected to be infected with highly infectious blood-borne pathogens.

Organization WH 2015. *Global technical strategy for malaria 2016-2030*, *World Health Organization*.

Organization WH 2018. Guide to G6PD deficiency rapid diagnostic testing to support *P. vivax* radical cure.

Organization WH 2020. World malaria report 2020, World Health Organization.

Ouattara AK, Yameogo P, Diarra B, Obiri-Yeboah D, Yonli A, Compaore TR*, et al.* 2016. 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,* 8(1).

Pal S, Myburgh J, Bansil P, Hann A, Robertson L, Gerth-Guyette E*, et al.* 2021. Reference and point-of-care testing for G6PD deficiency: Blood disorder interference, contrived specimens, and fingerstick equivalence and precision. *PloS ONE,* 16(9)**,** e0257560.

Pengboon P, Thamwarokun A, Changsri K, Kaset C &Chomean S 2019. Evaluation of quantitative biosensor for glucose-6-phosphate dehydrogenase activity detection. *PloS ONE,* 14(12)**,** e0226927.

Peters AL &van Noorden CJ 2017. Single cell cytochemistry illustrated by the demonstration of glucose-6-phosphate dehydrogenase deficiency in erythrocytes. *Histochemistry of Single Molecules. Springer*.

Segeja M, Mmbando BP, Kamugisha M, Akida J, Savaeli Z, Minja D*, et al.* 2008. Prevalence of glucose-6-phosphate dehydrogenase deficiency and haemoglobin S in high and moderate malaria transmission areas of Muheza, north-eastern Tanzania. *Tanzania Journal of Health Research,* 10(1)**,** 9-13.

Shitaye G, Gadisa E, Grignard L, Shumie G, Chali W, Menberu T*, et al.* 2018. Low and heterogeneous prevalence of glucose-6-phosphate dehydrogenase deficiency in different settings in Ethiopia using phenotyping and genotyping approaches. *Malaria Journal,* 17(1)**,** 281.

Spring R, Schlaack H, Rice M, Staat MA &Quinn CT 2018. Glucose-6-phosphate dehydrogenase deficiency in internationally adopted children. *Pediatric Blood & Cancer,* 65(5)**,** e26990.

Tabatabaei SM, Khorashad AS, Sakeni M, Raeisi A &Metanat Z 2015. Prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in southeast Iran: implications for malaria elimination. *The Journal of Infection in Developing Countries,* 9(03)**,** 289-97.

Taylor WR, Hoglund R, Peerawaranun P, Nguyen T, Hien T, Tarantola A*, et al.* 2021. Development of Weight and Age-based Dosing of Daily Primaquine for Radical Cure of *Vivax* Malaria.

Thielemans L, Gornsawun G, Hanboonkunupakarn B, Paw MK, Porn P, Moo PK*, et al.* 2018. Diagnostic performances of the fluorescent spot test for G6PD deficiency in newborns along the Thailand-Myanmar border: a cohort study. *Wellcome Open Research,* 3.

Thomas JE, Kang S, Wyatt CJ, Kim FS, Mangelsdorff AD &Weigel FK 2018. Glucose-6-Phosphate dehydrogenase deficiency is associated with cardiovascular disease in US Military centers. *Texas Heart Institute Journal,* 45(3)**,** 144-50.

Tsegaye A, Golassa L, Mamo H &Erko B 2014. Glucose-6-phosphate dehydrogenase deficiency among malaria suspects attending Gambella hospital, southwest Ethiopia. *Malaria Journal,* 13(1)**,** 438.

Tseghereda YG, Nganga JK, Kimang'a AN, Mehari TH &Weldemichael YG 2018. Glucose-6-phosphate dehydrogenase deficiency allelic variants and their prevalence in malaria patients in Eritrea. *The Pan African Medical Journal,* 31.

Valencia SH, Ocampo ID, Arce-Plata MI, Recht J &Arévalo-Herrera M 2016. Glucose-6-phosphate dehydrogenase deficiency prevalence and genetic variants in malaria endemic areas of Colombia. *Malaria Journal,* 15(1)**,** 291.

Williams O, Gbadero D, Edowhorhu G, Brearley A, Slusher T &Lund TC 2013. Glucose-6-phosphate dehydrogenase deficiency in Nigerian children. *PloS ONE,* 8(7)**,** e68800.

Zobrist S, Brito M, Garbin E, Monteiro WM, Clementino Freitas S, Macedo M*, et al.* 2021. Evaluation of a point-of-care diagnostic to identify glucose-6-phosphate dehydrogenase deficiency in Brazil. *PLoS Neglected Tropical Diseases,* 15(8)**,** e0009649.

11. Annexes

Annex- I. Information sheet (English version)

Addis Ababa University Postgraduate schedule

PI: Tassew Tefera

Name of organization: Addis Ababa University College of Health Science, School of Medicine, Department of Microbiology, Immunology and Parasitology information sheet.

Title: Prevalence of Glucose–6–phosphate dehydrogenase deficiency and distribution of its genetic variants among malaria suspected patients in Metehara Health Center, Eastern Ethiopia

Aim: To determine the magnitude of Glucose-6-phosphate dehydrogenase deficiency among attendants of selected health facilities in Metehara district, Ethiopia

Duration: For the purpose of G6PD rapid testing and blood sample collection you will spend only 10 to 20 minutes. The questionnaire will be filled and the consent form is signed.

Procedure to be followed: For this study to be successful we need your participation. If you are voluntary to participate, you are expected to understand and sign the informed consent. Socio demographic information is important and will be taken for the study. Venous blood and capillary blood sample will be collected and laboratory investigation will be done at onsite during specimen collection and EPHI after collection.

Risk: There is only minimal risk associated with sample collection and your time.

Expected benefits: As a participant of the study you are expected to give capillary and venous blood sampleand we will measurethe quantity of G6PD, Hemoglobin and G6PD/Hgb ratio using careSTART POCT S1 and Spectrophotometer methods. The genetic variants of G6PD deficient gene mutation will be performed by molecular method. The result will be discussed with the responsible physician but your personal information will not be disclosed to anyone. Only identification code will be used.

Confidentiality: All information that you give and the results from your specimen will be used for this study only. Limited number of professionals will have access to the information. All the information will be encoded in a computer and password protected.

Right: Refusal to participate in the study involves no penalty or loss of benefit to which you are otherwise entitled and participation is voluntary. You have the right to withhold information, decline to cooperate in the study and refuse provision of specimen.

Approval: This research project has got ethical clearance from the department research and ethics review committee (DRERC) of Addis Ababa University College of Health Science, School of Medicine, Department of Microbiology, Immunology and Parasitology and institutional review board of EPHI.

Whom to contact: If you have any question about this study you can communicate through the following address.

➔ Addis Ababa University College of Health Science School of Medicine, Department of Microbiology, Immunology and Parasitology

✓ Tel. --------------------- Fax.------------------- Email----------------------

➔ Principal Investigator : Tassew Tefera Phone : 0922406465

E-mail : tassewtefera@gmail.com

Annex-II Consent form for the study participants (English Version)

Code No. ----------------------

Name of the participant ------------------------

I have been informed about the study which is aimed in determining the magnitude of Glucose-6-phosphate dehydrogenase deficiency among attendants of selected health facilities in Metehara, Ethiopia. For this study blood sample is required. The aim and possible risk of the study were explained to me well. I have also informed that all the information contained in questionnaire is to be kept confidential. Moreover, I have been informed the rights to withdraw from study. It is therefore with full understanding I gave the informed consent voluntarily to the researcher to use my information and specimen for this study.

Participant's signature/Finger print ---------------------------

Name of data collector ------------------------------------ Sign --------------------- Date ---------------

Please contact us for any question or problems you may encounter during the study

Principal investigator: Tassew Tefera

Phone- 0922406465

E-mail:tassewtefera@gmail.com

Annex-III. Assent form for the study participants (English Version)

Code No. ----------------------

Name of study participant ---------------------------

Name of the participant's family or Guardian ------------------------

I have been informed about the study which is aimed in determining the magnitude of Glucose-6-phosphate dehydrogenase deficiency among attendants of selected health facilities in Metehara district, Ethiopia. For this study blood sample is required. The aim and possible risk of the study were explained to me well. I have also informed that all the information contained in questionnaire is to be kept confidential. Moreover, I have been informed the rights to withdraw from study. The study participant mentioned above who is not able to give the consent himself because he/she is younger than 18 years not allowed deciding on him/her. It is therefore with full understanding; by taking a full responsibility I gave my assent voluntarily to the researcher to use his/her information and specimen for this study.

Participant's signature/Finger print ---------------------------

Name of data collector ------------------------------------ Sign --------------------- Date ---------------

Please contact us for any question or problems you may encounter during the study

Principal investigator: Tassew Tefera

Phone- 0922406465

E-mail:tassewtefera@gmail.com

Annex-IV. Questionnaire (English version)

This questionnaire is prepared by Addis Ababa University, College of health science, Department of Medical Microbiology, Immunology and Parasitology graduate program student.

I thanks gratefully for your agreement to participate in this study. Now I am going to ask you interview questions and the interview is about general socio-demographic characteristics and clinical data. All of the answers you provide in this study will be kept confidential. The information you give me is very essential for this study. Therefore, I politely ask you to give me the right response.

Part I. Socio-demographic and Clinical information

1. Code no. --------------- Age…………… Sex: Male ☐ Female ☐
2. Address: Urban ☐ Rural ☐
3. Hemoglobin ---------------, G6PD---------------------, G6PD/Hgb ratio------------------
4. Have you ever been infected with malaria?

   A. Yes B. No
5. If yes for quest. 4, did you get any complication during malaria treatment or had been hospitalized?

   A. Yes B. No
6. Do you have any chronic health problems? A. Yes B. No
7. If yes for question no. 6, mention-------------------------
8. For only female participants, are you pregnant? A. Yes B. No
9. Currently, do you have sign and symptoms of malaria disease? A. Yes B. No
10. Which sign and symptoms do you have?

    A. Headache B. Fatigue C. Muscle and joint pain D. Shaking and chills

    E. Perspiration (sweeting) F. Anorexia (vomiting)
11. Malaria status of the participants? A. positive B. Negative C. Unknown
12. If positive for question no. 9, which species of plasmodium? A. *P. falciparum* B. *P. vivax* C. *P. ovalae* D. *P. malaria* E. mixed (*P.falciparum*/*vivax*)

Annex V. Information sheet (Amharic version)

አዲስ አበባ ዩኒቨርሲቲ የድህረ-ምረቃ ፕሮግራም

ጥናቱን የሚሰራዉ ሰዉ ስም፡ ጣሰዉ ተፈራ

ጥናቱን የሚያሰራዉ ተቋዋም፡ አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የህክምና ሳይንስ ት/ቤት የማይክሮባዮሎጂ፤ ኢሚዩኖሎጂ እና ፓራሳይቶሎጂ ትምህርት ክፍል

የጥናቱ ርዕስ፡ የ"Glucose-6-phosphate dehydrogenase" እጥረት ስርጭት በመተሃራ ጤና ጣቢያ በሚል ርዕስ ለሚደረገዉ ጥናት የተዘጋጀ መረጃ በአዲስ አበባ ዩኒቨርሲቲ በ"ፓራሳይቶሎጂ" የማስተርስ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልፅ ያልሆነዉን ይጠይቁ።

የጥናቱ ዓላማ፡ የ"Glucose-6-phosphate dehydrogenase" እጥረት እና የእርሱን ስነ ባህርይ የስርጭት መጠንን ማጥናት

እዚህ የሚቆዩበት ጊዜ፡ የተሰጠዎትን መጠይቅ ከሞሉና ከፈረሙ በኋላ ለጥናቱ የሚያስፈልገዉን የደም ናሙና በመስጠት ሂደቱን ይጨርሳሉ። ለዚህም ተግባር ከ 10 እስከ 20 ደቂቃ ብቻ ያጠፋሉ።

በዚህ ጥናት ሲሳተፉ የሚፈፅሟቸዉ ተግባራት፡ ለዚህ ጥናት መሳካትና ዉጤታማነት የእርስዎ አስተዋፅዖ በጣም ከፍተኛ ሲሆን ለመሳተፍ ፈቃደኛ በመሆንዎ እያመሰገንን፣ በጥናቱ ከመሳተፍዎ በፊት የፈቃደኝነት ዉሉን በደንብ አንብበዉ በመረዳት መፈረም አለብዎት። ዉሉን ከፈረሙ በኋላ በቃለ መጠይቁ ላይ የቀረበዉን ርስዎን የሚመለከት አጠቃላይ መረጃ በጥንቃቄ ከሞሉ በኋላ በሰለጠኑ ባለሙያዎች ከጣትዎ ላይ ሁለት ጠብታ ደም ተወስዶ የ G6PD እና Hemoglobin መጠን ይለካልዎታል፤ ለሞለኪዩላር ምርመራ DBS ናሙና ይሰበሰባል። በተጨማሪ 4 ሚሜ ደም ከክንድዎ ላይ በመርፌ ተቀድቶ ወደ ኢትዮጵያ ህብረተሰብ ጤና ኢንስትቲዩት ላቦራቶሪ ይወሰዳል።

በዚህ ጥናት መሳተፍ የሚያስከትለዉ ጉዳት፡ በጥናቱ መሳተፍ ናሙና ለመስጠት ከሚየጠፉት ጊዜ እና የደም ናሙና ሲወሰድ ከሚሰማዎት ቀላል ህመም ዉጪ ምንም ዓይነት ጉዳት አያስከትልም።

በዚህ ጥናት መሳተፍ የሚያስገኛቸዉ ጥቅሞች፡ የጥናቱ ተሳታፊ በመሆንዎ የሚያመጡት የደም ናሙና ጥራቱን በጠበቀ የአሰራር ሂደትና ልምድ ባላቸዉ ባለሙያዎች ሦስት ዓይነት የተሻሻሉ የላቦራቶሪ የምርመራ ዘዴዎችን በመጠቀም የG6PD and Hemoglobin ምርመራ ይሰራልዎታል።

የመረጃ ሚስጥራዊነት፡ እርስዎ የሚሰጡት መረጃና ከደም ናሙናዉ የሚገኘዉ ዉጤት ለዚህ ጥናት ዓላማ ብቻ ይዉላል። የምርመራ ዉጤትዎንም ለሚመለከተዉ ሃኪምዎ ብቻ በማሳዎቅ አስፈላጊዉን

ህክምና እንዲያገኙ እናደርጋለን። በስም ምትክ የሚስጥር ቁጥር ስለምንጠቀም ማንኛዉንም ዓይነት መረጃዎን ከሚመለከተዉ አካል ዉጪ ያለርስዎ ፈቃድ አናሳዉቅም።

የጥናቱ ተሳታፊ መብት፡ በዚህ ጥናት መሳተፍ የሚቻለዉ በራስ ተነሳሽነትና በሙሉ ፈቃደኝነት በመሆኑ፤ በማንኛዉም ጊዜና ሁኔታ መሳተፍ አልፈልግም ብሎ መተዉ ይቻላል። ጥናቱን አልሳተፍም ብሎ በመተዉ ምክንያት የሚደርስ ምንም ዓይነት ቅጣት፣ ኪሳራ እና ያልተገባ ዉንጀላ ወይም ነቀፌታ የለም። ፈቃደኛ ካልሆኑ መረጃዎትን የመደበቅ ወይም ያለመናገር፣ በጥናቱ ያለመሳተፍ አናም ለጥናቱ የሚያስፈልገዉን የደም ናሙና ያለመስጠት ሙሉ መብት አለዎት።

ስለ ጥናቱ ማረጋገጫ፡ ይህ ጥናት ከአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ ህክምና ሳይንስ ት/ቤት የማይክሮባዮሎጂ፤ ኢሚዩኖሎጂ እና ፓራሳይቶሎጂ ትምህርት ክፍል የምርምርና ህጋዊነት ኮሚቴ እዉቅናና ፈቃድ አግኝቷል። እንዲሁም ጥናቱ በሚሰራበት በመተሃራ ከተማ ጤና ተቐማት የበላይ ሀላፊዎችን በማስፈቀድ የሚሰራ ጥናት ነዉ።

ጥያቄ ቢኖረኝ/ችግር ቢያጋጥመኝ ምን ማድረግ እችላለሁ፡- ጥናቱን የተመለከተ ማንኛዉም ዓይነት ጥያቄ ካለዎት በሚከተሉት አድራሻዎች በመጠቀም መጠየቅ ይችላሉ፤

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ሳይንስ ት/ቤት የማይክሮባዮሎጂ፤ ኢሚዩኖሎጂ እና ፓራሳይቶሎጂ ትምህርት ክፍል

ስልክቁጥር፡ ----------------------- ፋክስ፡ ----------------------------- ኢ-ሜይል፡ ------------------------

ጥናቱን የሚሰራዉ፡ ጣሰዉ ተፈራ

ስልክ ቁጥር፡ 0922406465 ኢ-ሜይል፡tassewtefera@gmail.com

Annex-VI. Consent form for the study participants (Amharic Version)

የጥናቱ ተሳታፊዎች የስምምነት ቅጽ

የሚስጥር ቁጥር ----------------------

የተሳታፊዉ ስም ------------------------

እኔ ከዚህ በላይ ስሜ የተጠቀሰዉ የጥናቱ ተሳታፊ “Glucose-6-phosphate dehydrogenase እጥረት ስርጭት በመተሃራ ጤና ጣቢያ” በሚል ርዕስ ስለሚሰራዉ የምርምር ስራ አስፈላጊዉ መረጃ ሁሉ ተነግሮኛል። ለዚህ ጥናት የደም ናሙና መስጠት እንዳለብኝ፣ የምርምሩ ዓላማ ምን እንደሆነ እና በጥናቱ ምክንያት ሊከሰቱ የሚችሉ ጉዳቶች በዝርዝር ማብራሪያ ተሰጥቶኝ ተረድቻለሁ። በተጨማሪም ማንኛዉም ዓይነት ለዚህ ጥናት የሰጠሁት መረጃዬ በሚስጥር ተጠብቆ እንደሚያዝልኝ እና በፈለግሁት ግዜና ሁኔታ በጥናቱ መሳተፍ ካልፈለግሁ ማቋረጥ መብቴ እንደሆነ በግልፅ ተነግሮኛል። ስለዚህ የተሰጠኝን መረጃ መሰረት በማድረግ እና ዓላማዉንም በመረዳት በፈቃዴ የምሰጠዉን እኔን የሚመለከት መረጃ እና የደም ናሙና ለምርምር አገልግሎቱ እንዲያዉሉት ተስማምቼ መፍቀዴን በፊርማዬ አረጋግጠለሁ።

የተሳታፊዉ ፊርማ ---------------------------

የመረጃ ሰብሳቢዉ ስም ---------------------------   ፊርማ ---------------------   ቀን -------------------

ማንኛዉም ችግር ካጋጠመዎት በሚቀጥለዉ አድራሻዎች ያሳዉቁን

የጥናቱ ባለሙያ፡ ጣሰዉ ተፈራ

ስልክ ቁጥር፡ 0922406465

ኢ-ሜይል፡ tassewtefera@gmail.com

Annex-VII Assent form for the study participants (Amharic Version)

የጥናቱ ተሳታፊ ለሆኑ ህፃናት ወላጅ/አሳዳጊ የስምምነት ቅጽ

የሚስጥር ቁጥር ----------------------

የተሳታፊዉ ስም ------------------------

የተሳታፊዉ ወላጅ/አሳዳጊ ስም --------------------------

እኔ ከዚህ በላይ ስሜ የተጠቀሰዉ የጥናቱ ተሳታፊ ህፃን ወላጅ/አሳዳጊ ቤተሰብ "Glucose-6-phosphate dehydrogenase እጥረት ስርጭት በመተሃራ ጤና ጣቢያ" በሚል ርዕስ ስለሚሰራዉ የምርምር ስራ አስፈላጊዉ መረጃ ሁሉ ተነግሮኛል። ለዚህ ጥናት የደም ናሙና መስጠት እንዳለብኝ፣ የምርምሩ ዓላማ ምን እንደሆነ እና በጥናቱ ምክንያት ሊከሰቱ የሚችሉ ጉዳቶች በዝርዝር ማብራሪያ ተሰጥቶኝ ተረድቻለሁ። በተጨማሪም ማንኛዉም ዓይነት ለዚህ ጥናት የሰጠሁት መረጃዬ በሚስጥር ተጠብቆ እንደሚያዝልኝ እና በፈለግሁት ግዜና ሁኔታ በጥናቱ መሳተፍ ካልፈለግሁ ማቋረጥ መብቴ እንደሆነ በግልፅ ተነግሮኛል። ከላይ በስም የተጠቀሰዉ የጥናቱ ተሳታፊ ከ18 ዓመት እድሜ በታች በመሆኑና በራሱ ፈቃድ ለመሳተፍ ስምምነት መፈራረም ስለማይችል እኔ የእርሱ ወላጅ/አሳዳጊ ቤተሰብ በመሆኔ የተሰጠኝን መረጃ መሰረት በማድረግ እና ዓላማዉንም በመረዳት በፈቃዴ የምሰጠዉን ልጄን የሚመለከት መረጃ እና የደም ናሙና ለምርምር አገልግሎቱ እነዲያዉሉት ተስማምቼአለሁ።

የተሳታፊዉ ፊርማ ------------------------------

የመረጃ ሰብሳቢዉ ስም --------------------------- ፊርማ --------------------- ቀን -------------------

ማንኛዉም ችግር ካጋጠመዎት በሚቀጥለዉ አድራሻዎች ያሳዉቁን

የጥናቱ ባለሙያ፡ ጣሰዉተፈራ ስልክ ቁጥር፡ 0922406465

ኢ-ሜይል፡ tassewtefera@gmail.com

Annex-VIII. Questionnaire (Amharic version)

የተሳታፊ መጠየቆች

ይህ መጠይቅ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ሳይንስ ት/ቤት የማይክሮባዮሎጂ፤ ኢሚዩኖሎጂ እና ፓራሳይቶሎጂ ትምህርት ክፍል በሜዲካል ፓራሳይቶሎጂ የድህረ-ምረቃ ተማሪ የተዘጋጀ ነዉ። በቅድሚያ በዚህ ጥናት ላይ ለመሳተፍ ፈቃደኛ በመሆንዎ ላቅ ያለ ምስጋናዬን እያቀረብኩ ከዚህ በመቀጠል ለጥናቱ አስፈላጊ የሆኑ አጠቃላይ እርስዎን የሚገልጹ የስነ-ህዝብ እና የጤና ሁኔታ መረጃዎችን የሚያሳዩ ቃለ መጠይቆች ስላሉኝ በጥንቃቄ በመሙላት እንዲተባበሩኝ እጠይቃለሁ። እርስዎ የሚሰጡት መረጃ ለጥናቱ ወሳኝ በመሆኑ ትክክለኛዉን መረጃ በጥንቃቄ እንዲሰጡ እየጠየቅሁ እርስዎ የሚሰጡት ማንኛዉም ዓይነት መረጃ ሚስጥራዊነቱ የተጠበቀ እንደሚሆን ላረጋግጥልዎ እወዳለሁ።

የስነ- ህዝብ መረጃዎች እና የጤና ሁኔታ መረጃዎች

1. የሚስጥር ቁጥር --------------- ዕድሜ............... ፆታ: ወንድ ☐ ☐
2. አድራሻ: ከተማ ☐ ገጠር ☐
3. ሄሞግሎቢን ---------------, ግ6ፎዲ---------------------, ግ6ፎዲ/ሄሞግሎቢን ንፅፅር------------------
4. በወባ በሽታ ታመህ ታዉቃለህ?

   ሀ. አዎ ለ. አልታመምኩም
5. ለጥያቄ ቁጥር 4 መልስዎ አዎ ከሆነ፤ ሆስፒታል ዉስጥ ተኝቶ ለመታከም የሚያበቃ የከፋ ችግር ደርሶብዎት ነበር?

   ሀ. አዎ ለ. አልነበረም
6. ለረጅም ጊዜ የቆየ የጤና ችግር አለብዎት? ሀ. አዎ ለ. የለም
7. ለጥያቄ ቁጥር 6 መልስዎ አዎ ከሆነ፤ የጤና ችግሩን ይግለፁ-------------------------
8. አሁን የወባ በሽታ ምልክቶች እና ስሜት አለዎት ? ሀ. አዎ ለ. የለኝም
9. ተሳታፊዉ አሁን ያለበት የወባ ምርመራ ዉጤት ምንድነዉ? ሀ. በወባ በሽታ ተይዟል ለ.በወባ በሽታ አልተያዘም ሐ. አይታወቅም
10. ለጥያቄ ቁጥር 9 መልስዎ በወባ በሽታ ተይዟል ከሆነ፤ በየትኛዉ የወባ ዝርያ ነዉ የተያዘዉ?

    ሀ. *ፕላስሞዲየምፋልሲፓረም* ለ. ፕላስሞዲየምቫይቫክስ ሐ. *ፕላስሞዲየምኦቫሌ* ሠ. ፕላስሞዲየምማላሬ ረ. ድብልቅ(ፕ.ፋልሲፓረም እና ፕ.ቫይቫክስ)

Annex IX. Information sheet (Afaan Oromoo version)

Yuunivarsiti Finfinneeti Sagantaa Eebbaa digirii lamaffaa

Maqaa nama qorannoo gaggeessuu: Xaasoo Tafarraa

Maqaa dhaabbata Qorannicha adeemsisuu: Yuunivarsitii Finfinneeti, Kolleejjii saayinsii Fayyaati, Dipaartimeentii Meedikaala Maakiroobaayoolojjii, Imunoolojjii fi Paraasaatoolojjiiti.

Mata-duree Qorannoo: Tatamsa'ina hanqina Glucose-6-phosphate dehydrogenase naannoo Matahaaraati dhaabbilee fayyaa filataman keessati mata-duree jedhuun qorannoo hojjetamuuf odeeffannoo qophaa'e.

Yuunivarsitii Finfinneeti kutaa Paraasaayitoolojjiiti qorannoo eebbaa digirii lamattaaf qophaa'e irraatti akka hirmaattan affeeramtanittu. Qorannoo kanarraatti hirmaachuun dura barreeffama kanati aanee jiru xiyyeeffannoon dubbisuun kan isiniif ifa hin taane gaafachuu dandeessu.

Kaayyoo Qorannoo: Hanga tatamsa'ina hanqina"Glucose-6-phosphate dehydrogenase" beekuuf.

Hanga yeroo turtii as turan: Gaaffii bifa barreeffaman isiniif kenname erga guutanii fi mallatteessitanii booda qorannichaaf kan barbaachisu saamuda dhiiga kennuun adeemsa hojii kanaa xumurtu. Hojii kanaaf daqiqaa 10 hanga 20 qofa isinitti fudhata.

Qorannoo kanarratti yemmuu hirmaattan gocha raawataman: Milkaa'ina fi bu'a qabeessummaa qorannoo kanaaf deeggarsi naaf taasiftan baayyee guddaa yoo ta'u, hirmaachuu keessaniif eeyyamamaa waan taataniif isin galatteeffachaa, qorannicha irraatti hirmaachuun dura foormii waligaltee sirriitti dubbisuun erga hubattaniin booda mallatteessuun isinirraa eegama. Kanatti fufuun gaaffii odeeffannoo wali galaa waa'ee keessan of eeggannoon erga guuttaniin booda ogeessota leenji'aniin quba keessan irraa copa dhiigaa lama (two drops of blood) fudhachuun qorannoon G6PD fi Hemoglobin isiniif goona. Qorannoo molakiyulariif saamudni dhiigaa DBS dhaan fudhatama. Dabalataanis saamuda dhiigaa mililitirii 4 ciqilee keessan irraa marfeedhaan isinirraa fudhatamee gara dhaabbatta laboratoorii haawasummaa Itiyoophiyaatti ergama.

Qoranno kanarratti hirmaachuun miidhaa qabu: Qorannoo kanarraatti hirmaachuun saamuda dhiigaa kennuuf yeroo keessan balleessitanii fi saamuda dhiigaa yeroo kennitan dhukkubii salphaa isinitti dhagahamu ala miidhaa kamiyyuu hin qabu.

Qorannoo kana irratti hirmachuun bu'aa inni argamsisuu

Hirmaata qorannoo kana tahuu keessanin saamudni dhiigaa isin kennitan akkataa qulqulinaa isa eegateef ogeessotaa muuxannoo qabanin mala qorannoo laaboratoorii fooyya'aa tahe gosa sadii fayyadamuun qorannoo G6PD fi Hemoogilobini isiniif goona.

Iccitii ragaa eegamuu qabu

Ragaaf saamuda dhiigaa isin nuf kennitan irraa bu'aan qorannoo argamuu kamuu kaayyoo qorannaa kana duwwaaf kan ooludha. Bu'aan qorannoo keessanis Hakimii keessaniif qofa beeksisuun tajaajila barbaachisaa akka argattan isiniif goona. Bakka maqaa keessani lakkoofsa iccitii waan fayyadamnuuf ragaa qorannaa irraa argamuu kamiyyuu qaama dhimmi ilaalu malee eeyyamaa keessanin alatti hin beeksifnu.

Mirga hirmaataa qorannoo kana: Qorannoo kanarratti hirmaachuun kan danda'amu kaka'umsa mataa ofii fi eeyyama keessan guutuun akka ta'e, yeroo fi haala kamiyyuu keessatti hirmaachuu hin barbaadu jechuun keessaa of baasuun ni danda'ama. Qorannoo kana sababii addaan kutaniif adabbiin homtuu hin jiru, kasaaruu fi maqa xureessi hin qabu. Eeyyamamaa yoo hin taane odeeffannoo dhoksuu ykn dubbachuu dhiisuu, qorannoorratti hirmaachuu dhiisuu akkasumas saamuda dhiigaa qorannoof barbaachisu mirga kennuu diduu qabdu.

Waa'ee Qorannoo mirkanneeffannaa: Qorannoon KunYuunivarsitii Finfinneeti, Kolleejjii saayinsii Fayyaati, Dipaartimeentii Meedikaala Maakiroobaayoolojjii, Imunoolojjii fi Paraasaatoolojjiiti koree qorannoo fi seeraa irraa beekamtii fi eeyyama argateera. Akkasumas bakka qorannoon itti gaggeeffamu magaalaa Matahaaraati waajira eegumsa fayyaa eeyyamsiisuun qorannoo hojjetamudha.

Gaaffii yoon qabaadhe/Rakkoon yoo na mudate maal gochuun qabaadha: Qorannicha ilaalchisee gaaffii kamiyyuu yoo qabaattan teessoo armaan gadii fayadamuun gaafachuu

Lakk. Bilbilaa: ______________**Faksii:**_______________**E-mail**: ____________________

Maqaa nama qorannoo gageessuu: Xaasoo Tafarraa

Lakk. Bilbilaa: 0922406465 **E-mail**: tassewtefera@gmail.com

Annex- X. Consent form for the study participants (Afaan Oromoo Version)

Foormii walii galtee hirmaatota waggaati 18 oli ta'ani

Lakk. Iccitii____________________

Maqaa hirmaataa________________

Ani maqaan koo armaan olitti kan ibsame hirmaataa qorannoo kan mata dureen isaa "Tatamsa'ina hanqina Glucose-6-phosphate dehydrogenase naannoo matahaaraatti dhaabbilee fayyaa filataman keessati" jedhuun waa'ee faayidaa qorannoo taasifamuu odeeffannoo barbaachisaa hunda dhagaheera. Qorannoo kanaaf saamuda dhiigaa kennuu akkan qabu, kaayyoo qorannichaa maal akka ta'e fi sababa qorannichaan miidhaa narra gahuu danda'u tokko tokkoon naaf ibsamee hubadheera. Dabalataanis odeeffannoo kamiyyuu qorannoo kanaaf kenname iccitiin isaa eegamaa akka ta'e fi yeroon barbaadee fi haala kamiyyuu keessatti qorannichati hirmaachuu keessa mirga addaan kutuu akkan qabu ifaan natti himameera. Kanaafuu haala odeeffannoo naaf kennameen kaayyoo isaa hubadhee eeyyama kiyyaan odeeffannoo barbaachisuu fi saamuda dhiigaa qorannoof barbaachisu akka qorannoof oolchan irratti walii galee eeyyamamaa ta'uu koo mallattoo kootiin mirkanneessa.

Mallattoo hirmaataa________________________

Maqaa nama odeeffannoo gaafatuu____________mallattoo__________Guyyaa_________

Rakkoon kamiyyuu yoo isin mudate teessoo armaan gadiitin nu beeksisaa:

Maqaa qorannoo gaggeessu: Xaasoo Tafarraa

Lakk. Bilbilaa: 0922406465

E-mail: tassewtefera@gmail.com

Annex- XI. Asset form for the study participants (Afaan Oromoo Version)

Foormii walii galtee Maatii ykn Guddiftuu hirmaatota waggaa 18 gadi

Lakk. Iccitii___________________________________

Maqaa hirmaataa_______________________________

Maqaa maatii/ guddiftuu hirmaataa_______________

Ani armaan oliiti maqaan koo kan ibsame maatii/guddiftuu mucaa hirmaataa mata duree "Tatamsa'ina hanqina Glucose-6-phosphate dehydrogenase naannoo Matahaaraatti dhaabbilee fayyaa filataman keessati" jedhuun waa'ee qorannoo taasifamuu odeeffannoo dhagaheera. Qorannoo kanaaf saamuda dhiigaa kennuu akka qabu, kaayyoo qorannichaa maal akka ta'e fi sababa qorannichaan miidhaa irra gahuu danda'u tokko tokkoon naaf ibsamee hubadheera. Dabalataanis odeeffannoo kamiyyuu qorannoo kanaaf kenname iccitiin isaa eegamaa akka ta'e fi yeroon barbaadee fi haala kamiyyuu keessatti qorannichati hirmaachuu keessa mirga addaan kutuu akka qabu ifaan natti himameera. Armaan olitti hirmaataan maqaan isaa ibsame waggaa 18 gadi waan ta'eef fedhii isaan hirmaachuuf walii galtee mallatteessuu waan hin dandeenyeef maatii/guddiftuu isaa ta'ee haala odeeffannoo naaf kennameen kaayyoo isaa hubadhee eeyyama kootiin mucaan koo odeeffannoo barbaachisuu fi saamuda dhiigaa qorannoo barbaachisuuf akka oolchitan walii gallee, eeyyamamaa ta'uu koo mallattoo kootiin mirkanneessa.

Mallattoo hirmaataa____________________

Maqaa nama odeeffannoo gaafatuu______________mallattoo_________Guyyaa________

Rakkoon kamiyyuu yoo isin quuname teessoo armaan gadiin nu beeksisa:

Maqaa nama qorannicha gaggeessuu: Xaasoo Tafarraa

Lakk. Bilbilaa: 0922406465

E-mail:tassewtefera@gmail.com

Annex-XII. Questionnaire (Afaan Oromoo Version)

Hiikkaa gaaffii garagalcha Afaan Oromoo

Foormii gaaffii kanaa Yuunivarsitii Finfinneeti, Kolleejjii saayinsii Fayyaati, Dipaartimeentii Meedikaala Maakiroobaayoolojjii, Imunoolojjii fi Paraasaatoolojjiiti baratаа digirii lamaattaatiin qophaa'e.

Duraan dursee qorannoo kanarraatti hirmaachuuf eeyyamamaa ta'uu keessaniif galatooma isiniin jechaa, kanatti aansuudhaan qorannoo kanaaf barbaachisaa kan ta'e odeeffannoo haala walii gala jireenyaa fi haala fayyaa keessanii mul'isu gaaffii salphaa waan qabuuf of eeggannoon akka naaf guuttan kabajaan isin gaafadha. Odeeffannoon isin naaf kennitan qorannoo kanaaf baayyee murteessaa waan ta'eef deebii sirrii hubannaan akka naaf deebistan isin gaafachaa odeeffannoo isin naaf kennitan kamiyyuu icciitiin isaa eegamaa ta'uu isiniif mirkanneessa.

Gaaffii haala jireenyaa fi odeeffannoo haala fayyaa

1. Lakk. Iccittii___________ Umurii__________ Saala: A. Dhiira B. Durba
2. Teessoo: A. Magaalaa B. Baadiyyaa
3. Hemoglobin___________, G6PD_____________, Ratio G6PD/Hemoglobin__________
4. Dhukkubni busaa si qabee beekaa? A. Eeyyee B. Lakki
5. Gaaffii lakk. 4 deebiin eeyyee yoo ta'e, rakkoo sadarkaa hospitaala keessa ciisaani yaalamuu isinirraan gahee turee? A. Eeyyee B. Lakki
6. Yeroo dheeraa kan isinirra ture rakkoo fayyaa qabduu? A. Eeyyeen B.Lakki
7. Gaaffii lakk. 6 deebiin eeyyeen yoo ta'e, rakkoo fayyaa isin quuname ibsa____________
8. Hirmaattota dubartootaa qofaaf; Ulfa dhaa? A. Eeyyee B. Lakki
9. Amma mallattoo busaa qabduu? A. Eeyyeen B. Lakki
10. Bu'aa qorannoo busaa. A. pozativii B. Nageetivii C. Hin beekamu
11. Gaaffii lakk.10 deebiin pozativii yoo ta'e, gosa plasmodium isa kami?
    A. P. falciparum B. P. Vivax C. P. ovale D. Makaa

Annex XIII. Procedures for laboratory investigations of G6PD

1. Procedure for careSTART™ POCT S1 using capillary blood to measure G6PD activity

1.1. Specimen collection

1.1.1. Wash hands with warm water and soap, then dry them completely. Rubbing fingers helps blood being discharged easily;

1.1.2. Follow the lancet's instructions for use;

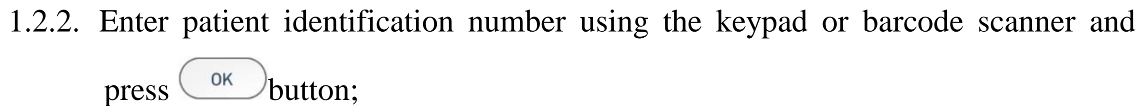
1.1.3. Hold the lancet against the fingertip. Press the release button. When you hear a click, the puncture is complete.

1.1.4. Discard a used lancet into safety box.

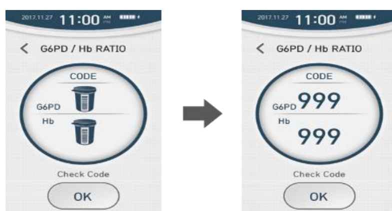
1.2. Measure G6PD/Hb Ratio or G6PD activity

1.2.1. Select button for starting measurement on the main menu screen;

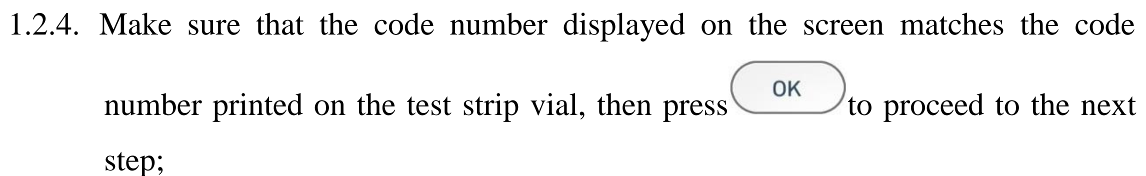
1.2.2. Enter patient identification number using the keypad or barcode scanner and press button;



1.2.3. Scan G6PD and Hb QR code printed on the test strip vial. If the QR code is read successfully, the code number will be displayed on the screen;

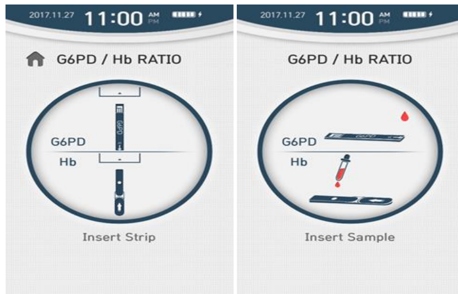


1.2.4. Make sure that the code number displayed on the screen matches the code number printed on the test strip vial, then press to proceed to the next step;

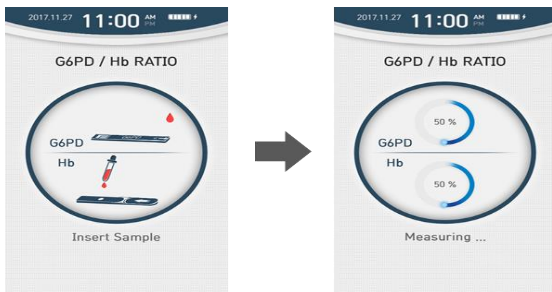


1.2.5. Take a new careSTART™ POCT S1 G6PD strip and Hb strip from the vial and close the lid after taking out the strip;

1.2.6. Insert the G6PD test strip into the G6PD test strip port, with the ‘G6PD’ label facing upwards. Gently push the test strip into the port until it stops. Then insert the Hb test strip into the Hb test strip port. If the strips are inserted normally, the buzzer will sound and ‘Insert Sample’ screen will be displayed.



1.2.7. Obtain a blood sample using the lancing device. Apply the blood sample into strips. If the sample is loaded successfully, the measurement will begin with a beep sound and the progress bar will be displayed as shown below. When the progress bar reaches 100%, the measurement is completed.



1.2.8. When the measurement is completed, the result is displayed on the screen and stored to the memory.

1.2.9. Press button to return to the main screen.

2. Procedure for Genotyping of G6PD variants by molecular method

PCR reactions were performed to amplify exons 4-11 of the *G6PD* gene to characterize the genotypic mutations.

- **2.1.** DNA was extracted from DBS samples using the PureLink™ Genomic DNA Mini Kit according to manufacturer's recommendations (Invitrogen ™).
- **2.2.** All PCR reactions were performed to a final volume of 20 µl, using 10 µl of 2xMaxima SYBR green PCR Master Mix according to manufacturer's recommendations, 2 µl of DNA and specific primers for each region.
- **2.3.** The PCR conditions used to amplify the three fragments are: 94 °C/3 min, 38 cycles of 94 °C/30 s, 55-58 °C/30 s, and 72 °C/60 s and final extension of 72 °C/6 min.
- **2.4.** Amplified PCR products were separated by gel electrophoresis with 1.5% agarose gel at 120 V for 2.00 hours.
- **2.5.** Amplified PCR products were purified and sequenced on an ABI 3730xl DNA analyzer based on using published protocols.
- **2.6.** Sequences were analyzed BioEdit. All sequences were aligned to the NCBI reference sequence (NG_009015.2) to verify the specificity of the PCR products.
- **2.7.** Samples with poor sequencing quality or showed singleton mutations were re-amplified and sequenced. Frequency of all detected mutations will be compared between G6PD normal and deficient patient samples.

Annex XIV. Procedures for malaria diagnostic methods

1. Procedure for malaria smear microscopy

For malaria microscopy laboratory technique thick and thin blood films can be used. Thick blood films are more sensitive in detecting malaria parasites because the blood is concentrated, allowing a greater volume of blood to be examined. Thin blood film is good for species identification of plasmodium species.

1.1. Select clean or new slides, label them with lead pencil on the frosted side of the slide.

1.2. Select appropriate site (soft finger or heel for infants) for puncture, clean with 70% alcohol. Puncture, wipe away the first drop with clean gauze.

1.3. Collect the second drop by touching it with the side of the slides. Make thick and thin blood films either separately or on the same slide.

1.4. Thick and thin blood films were done correctly according to the standard procedure.

1.5. Smears were air dried and thin smears were fixed with methanol alcohol for 30 seconds.

1.6. Then thick and thin smears were stained with 10% geimsa reagent for 10 minutes and wash with clean water, clean the underside of the slides with gauze, then dry stained slides with air.

1.7. The stained smears were investigated with a light microscope by high power magnification (100x) objective to detect the presence of malaria parasites. Thick film preparations were examined first by high power magnification (100x) objective for the presence of parasites.

1.8. The results reported with appropriate grading.

2. Procedure for malaria diagnosis using RDT method

2.1. Make a gentle finger prick of the disinfected site of the not calloused finger with a sterile lancet. Discard the used lancet to appropriate container and by applying gentle pressure remove the first drop of blood.

2.2. Using the blood collection device (pipette, inverted cup or capillary tube) Collect 5µl of blood. After pricking and collecting blood, apply a dry cotton wool at the puncture site to stop the bleeding. Discard the blood collection device in the box for infectious waste.

2.3. Apply the collected capillary whole blood specimen to the specimen well (circle) of the test device.

2.4. Hold the buffer bottle at 90° (perpendicular) to the test device and not touching the specimen well to avoid contamination. Add 3 drops (90µl) of the buffer into the buffer well (square) of the test device.

2.5. Adjust the timer 15 minutes and read the results between 15-30 minutes. Do not read the result after 30 minutes.

2.6. Result interpretation

A. The presence of only one colored band ("C" Control line) within the result window indicates a negative result.

B. The presence of two colored bands ("C" Control line and "P.f" *Plasmodium falciparum* line) within the result window, no matter which band appears first, indicates a P.f. positive result.

C. The presence of two colored bands ("C" Control line and "P.v" *Plasmodium vivax* line) within the result window, no matter which band appears first, indicates a P.v positive result.

D. The presence of three colored bands ("C" Control line, "P.f" *Plasmodium falciparum* line and "P.v" *Plasmodium vivax* line) within the result window, no matter which band appears first, indicates a mixed P.f. and P. v positive result.

E. If the control band ("C" Control line) is not visible within the result window, the result is considered invalid. The directions may not have been followed correctly or the test device may have deteriorated. Re-test with a new specimen and a new test device.

12. Declaration

I, the undersigned candidate, declare that this M.Sc. thesis is my original work, that it has not been previously submitted for a degree at this or any other university, and that all sources of materials utilized in the thesis have been properly acknowledged.

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