G6PD Deficiency and Its Correlation to Anaemia Among People Living in High Endemic Malaria Area, Nimbokrang, Papua, Indonesia

Muhammad F. Rokhmad[1(✉)], Yustinus Maladan[1], Setyo Adiningsih[1(✉)], Mirna Widiyanti[1], and Ivon Ayomi[2]

[1] National Research and Innovation Agency of Indonesia, Jakarta, Indonesia
{muha289,sety015}@brin.go.id
[2] Public Health Laboratory, Indonesia Ministry of Health, Papua, Indonesia

Abstract. G6PD deficiency is an X-linked enzymopathy and is the most prevalent genetic disorder in the world, affecting 500 million people, and it frequently causes hemolytic anemia. This genetic disorder is often associated with malaria because of its high prevalence in malaria endemic areas such as Africa, Southeast Asia, and the Pacific region. This research aimed to observe the G6PD deficiency prevalence and its correlation with anemia among people living in Nimbokrang District, since it is one districts with the highest malaria prevalence in Papua, and in Jayapura province is the top-five provinces with highest malaria case in the given province. This research was cross sectional, and 200 participants were randomly chosen and 1 cc of venous blood sample was taken from each participant. Blood samples were then analyzed qualitatively for G6PD deficiency using RDT (HumasisTM) and quantitatively for Hemoglobin level using HemoCue Hb801. The collected data were analyzed using SPSS version 22 for Fischer exact analysis, and a P value < 0.05 was statistically significant. It revealed that 42 out of 200 participants were deficient in G6PD, 19 of whom were males, and the rest were females. The result of Fischer exact analysis showed high significant correlation between G6PD deficiency and low hemoglobin level, and predominantly among female participants. G6PD deficiency was quite prevalent among participants, with a prevalence of 21%, mostly dominated by females with mild to asymptomatic anemia and their correlation was statistically significant with 0.002 of P value.

Keywords: G6PD · Malaria · asymptomatic anemia · hemolytic anemia · deficiency

1 Introduction

G6PD deficiency is the most common hereditary X-linked disorder in the state of enzymopathy and the world's the current population reaches more than 500 million people [1–3]. This genetic disorder is caused by a mutation that leads to an amino acid substitution, and because it is located on the X-chromosome, males are more likely to be affected than females, although females are typically carriers. Generally, the genotypes

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of G6PD deficiency in males only for two so forth, hemizygous normal and the rest is hemizygous deficient. However, there are three different genotypes found in female which are homozygous normal, homozygous deficient and heterozygous. As a result of deactivation in one of the X-chromosomes, known as Lyonization [4, 5], the heterozygous females are, on average, genetic mosaics, meaning that half of their erythrocyte are G6PD normal and the other half are G6PD deficient [1, 6, 7]. In this condition, most females will express normal G6PD activity, but on the other hand the rest will overlap showing clinical symptoms consistent with homozygous G6PD deficiency. Interestingly, G6PD deficiency will remain silent and show no clinical symptoms for most of a person's life, and it is never completely cured, and if it were, it would be lethal. Furthermore, in its steady state, the consequences are hardly noticeable, unless the deficient individuals are exposed to oxidative damage triggered by the consumption of quinone-containing chemical substance such as primaquine and dapsone [1, 2].

G6PD is a housekeeping enzyme and it is ubiquitously expressed in all body cells and primarily catalyzes the oxidation of glucose 6-phosphate (G6P) to phosphoglucono-δ-lactone, in turn which it has a crucial role in the pentose phosphate pathway which will help synthesizing the formation of the NADPH molecule which is widely involved in the metabolism of many drugs and xenobiotics compounds as electron donors and ultimately provides protection for body cells to assist fighting free radicals. Despite becoming abundant, the most particular existence of G6PD found in RBC, since it cannot to synthesis protein by itself and exquisitely susceptible to oxidative stress from exogenous oxidative agents as well [1, 3, 7]. Likewise, G6PD plays essential role in maintaining the cell integrity of RBC, and in deficient individual's RBC's life span will decrease and frequently found to be less than 120 days [1, 8, 9].

It has close geographical correlation malaria, since this enzymopathy is commonly found in people in areas with a high prevalence of malaria and appears to provide plausible protection against plasmodia infection [3, 10–12]. *Plasmodium falciparum* was long believed to be the selective agent for G6PD deficiency, but recent evidence suggests *Plasmodium vivax*, where present, may have also played an important role [1]. The immune system detects infected red blood corpuscles as abnormal and remove them, thereby preventing the parasite from advancing to the next stage of its erythrocytic phase [1, 13]. Patients with G6PD deficiency are required to take primaquine when a severe and acute infection occurs, despite the fact that G6PDd has a slight protective effect against malaria. G6PD seems providing such crossroad of hematology, pharmacogenetics, and malariology, and indeed there is quite a triangular relationship. Firstly, malarial plasmodia select for a group of humans who are G6PD deficient, therefore primaquine is a quite potent anti-malarial but it is dangerous for those G6PD deficient mutant that malaria has selected for [2, 11, 14].

Papua province has long been known for its malaria high endemicity in Indonesia along with West-Papua and East Nusa Tenggara province respectively, and currently one of the top-five regencies having highest malaria prevalence is Jayapura [5]. In the case of the Jayapura regency, a few districts, such as Nimbokrang District, have a relatively high incidence of malaria, even though various anti-malarial strategies have already been implemented. On the other hand, Nimbokrang's mixed settlement community and status as one of the oldest transmigrant settings in the northern Papua region make it a

fascinating location for studying malaria with a focus on G6PD deficiency. Additionally, this district has been selected for multiple malaria studies. Local native Papuans have been exposed to malaria for generations and have likely developed an adequate immune response and G6PD deficiency, but in regard to the new arrivals, this remains a mystery that is quite intriguing to investigate. This study aimed to determine the prevalence of G6PD deficiency and its association with anemia among Nimbokrang residents.

2 Material and Methods

2.1 Study Design and Participants

This study was designed as cross-sectional and the data collection was conducted in October 2021, including sociodemographic data, anaemia status, malaria infection, and G6PD deficiency as well. Nimbokrang district has a mixed population containing local native Papuan and other new arrival (transmigrants) and the area was previously part of the tropical rain forest. This district carries one of the highest malaria burdens within the Jayapura regency and the peak season for malaria occurs around October to March. The total 200 participants were selected randomly based on the household list of recipients of LLINs mosquito nets from the Jayapura district health office provided by the Nimbokrang Public Health Centre, which then underwent identification, and numbered.

2.2 Data Collection

Following written informed consent, a structured questionnaire and medical examination using malaria, and G6PD deficiency rapid diagnostic test (RDT) (CareStart™ Malaria RDT & Humasis G6PD) and hemoglobin (Hb) measurement (Hemocue 201, Angelholm, Sweden) were conducted on site, and all those measurements comprised by 1 cc the venous blood sampling, then the rest of the sampled blood transferred into an EDTA container (Zhejiang Gongdong Medical technology Ltd.). Those participants who were positively detected with malaria, G6PD deficiency, and anemia status respectively, informed directly then underwent referred to the closest medical facility for further treatment. Data then underwent recorded then digitally stored in team's personal computer.

2.3 Statistical Analysis

The data of demographic, and clinical characteristics of participant were analyzed statistically (Fisher exact test) to determine the correlation of each variable using IBM SPSS Statistics 26.

2.4 Ethical Approval

The study was approved by the Ethics Review Committee of National Institute of Health Research and Development of Indonesian Ministry of Health (LB.02.01/2/KE.596/2021). Written informed consent was distributed and recollected from all participants of their legal guardians prior to enrolment and in addition written assent was collected from all minors above the age of 5 years.

Table 1. Participants characteristics.

Variables	Frequency (n = 72)	Percent (%)
Sex		
Male	30	41.7
Female	42	58.3
Age groups (years)		
5–11	4	5.6
12–25	21	29.2
26–45	23	31.9
46–65	22	30.6
Above 65	2	2.8
Haemoglobin level		
Normal	23	31.9
Mild anaemia	18	25.0
Moderate anaemia	29	40.3
Severe anaemia	2	2.8
Malaria test		
Positive	7	9.7
Negative	65	90.3
G6PD test		
Positive	33	45.8
Negative	39	54.2
Total	72	100.0

3 Results

3.1 Demographic and Clinical Characteristics of Study Participants

The given Table 1 shows the shorted data containing participant study number that is completed with several features such as sex, age, hemoglobin (g/dL), malaria status, and G6PD deficiency (both malaria and G6PD were qualitatively measured using RDTs).

The research findings revealed that participants with G6PD deficiency were quite prevalent, and its qualitative prevalence reached at value 21%. Female G6PD deficiency predominates over male G6PD deficiency in the given study area, and nearly all deficient females have lower hemoglobin according to the WHO standard, which states that the normal Hb range for adult females is between 11.0 and 14.0 g/dL (Table 2). Even

Table 2. Association of gender, malaria infection, and hemoglobin level with G6PD among participants (n = 197)

Variables	G6PD test		p	Odd Ratio	95% Confidence Interval	
	Positive	Negative			Lower	Upper
Gender						
Male	58 (75.3%)	19 (24.7%)	0.228	0.685	0.342	1.372
Female	98 (81.7%)	22 (18.3%)				
Malaria infection test						
Positive	13 (86.7%)	2 (13.3%)	0.741	1.773	0.384	8.189
Negative	143 (78.6%)	39 (21.4%)				
Haemoglobin level						
Anemia	51 (68.0%)	24 (32.0%)	**0,004***	0.344	0.170	0.697
Normal	105 (86.1%)	17 (13.9%)				

*Statistically significant with p-value < 0.05

though some participants are G6PD-deficient in both males and females, it is unlikely that they will always develop anemia, and vice versa, it is possible that G6PD-normal participants will develop anemia under certain conditions. Malarial infection provides vital contribution to the state of anemia as well. Focusing at the Asterix-study number, participant who coincidentally infected by plasmodium seems to have bigger probability to develop anemia, despite not becoming a G6PD deficient. Non-*Plasmodium falciparum* (PAN) in this case contributes to the bigger scale for participant to have lower Hb concentration.

According to the given table for crosstab (fisher exact test) it is quite surprising that participant with G6PD deficiency have a risk factor for anemia that is only 0.3 times higher than those with normal G6PD levels. Those with normal G6PD levels have a risk factor for anemia that is 3,3 times lower.

4 Discussion

It has been long known and proofed by scientific studies that G6PD deficiency is geographically distributed in concordance with the spatial distribution of malaria and frequently presents when plasmodia infection is common [1–3, 15]. According to this research the G6PD deficiency prevalence obtained was valued 21% as measured by a qualitative rapid diagnostic test. This result partially compensates for the lack of primary data regarding the prevalence of deficient individuals in Nimbokrang District's population. However, in the other side, this finding encourages more challenging efforts to be made in the future to conduct more in-depth research since the outcome of qualitative measurement is deficient. Despite being good in detecting G6PD deficiency in males without need for laboratory expertise, the result validity is quite doubtable since RDTs

usually work well in each circumstance where humidity and temperature plays vital role in determining the final test result. Furthermore, it is common inconvenience states that qualitative methods are insufficiently sensitive to detect females with G6PD heterozygous deficiency, as this genotype produces two distinct RBC groups, one normal and the other deficient [1, 4, 16].

As previous studies have revealed that deficiency of G6PD does not always result in severe anemia and is typically asymptomatic until the individual is exposed to substances such as quinones (primaquine, chloroquine) [1, 7, 15]. The state of immunity and personal stamina plays an important role and seems to be a vital determinant for individuals with G6PD deficiency conditions to develop anemia from mild to severe. Mutation variants in the gene that encodes the G6PD enzyme in turn will also play an important role whether future G6PD-deficient individuals will experience anemia when exposed to oxidative substances or infected with malaria.

Malaria is one of the major causes of anemia in the endemic areas, and the anemia cases found in the results of this study seemed to be caused by more complex conditions, such as fatigue, menstruation, diet, and malaria infection [17–19]. This is in line with the previous theory that geographically the prevalence of G6PD deficiency will be directly proportional to the prevalence of malaria in an area. G6PD enzyme deficiency is a molecular defense where in the absence of this housekeeping enzyme, the individual will have temporary immunity against mild malaria infection, because usually red blood cells in deficient individuals will be short-lived because they are considered abnormal by the immune system and will be recycled immediately, and thus the opportunity for plasmodium to metagenesis to the sexual phase is automatically stopped [17].

Malarial infection, especially recurrent infections, stimulates erythroid hyperplasia which in turn increasing folates requirement and can lead to folate depletion and megaloblastic anemia, which usually profound during women pregnancies, where folate levels someway can be misleadingly high [20]. Iron deficiency and malaria often coincide in severely anemic subjects. It has been postulated that malaria contribute to iron depletion through decreasing intake during anorexia, reducing absorption and causing lost through hemoglobinuria [21]. In this study, malaria infection contributed more to anemia than congenital conditions such as G6PD deficiency. Statistically, however, there was a strong correlation between anemia and G6PD deficiency. However, it is necessary to remember that anemia is a potent confounding factor increasing the probability of false-positive diagnoses [17]. This given circumstance decreases the total number of RBCs, and therefore the level of G6PD enzyme level per volume of blood. Whereas increased proportion of reticulocytes following malaria infection can direct to false negative as reticulocytes hit the highest G6PD activity levels [1]. Consequently, a comprehensive examination of this case is required, for instance by employing a molecular approach to identify the G6PD mutation variant with a high prevalence in the Nimbokrang district of Jayapura, Papua New Guinea. In addition, it is necessary to research the ethnicity-related characteristics in the given field of study.

5 Conclusion

According to the findings of the study, the prevalence of G6PD deficiency in Papua New Guinea is quite high (21%). Despite a statistically significant correlation between

G6PD deficiency and hemoglobin level, it was discovered in this study that G6PD-deficient participants did not necessarily develop anemia. Since the qualitative method for detecting G6PD deficiency has several flaws in the final test result, the molecular approach appears to be a promising alternative.

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