

Original Research Article

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## Prevalence of G6PD Deficiency among the Malaria Patients in Urban Kolkata, India - A Clinic Based Study

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### ABSTRACT

#### Keywords

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#### Article Info

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The estimation of G6PD deficiency (G6PDd) is an important aspect in the current situation of malaria elimination which requires use of primaquine to prevent transmission of *P. falciparum* and relapse of *P. vivax*. Long term use of primaquine can induce acute hemolytic anemia in G6PD deficient cases. A very little information is available about the prevalence of G6PDd among the malaria positive cases from India. The present study was designed to evaluate the G6PDd among the malaria positive patients attending the Calcutta School of Tropical Medicine. A total of 583 blood samples were collected from diagnosed malaria cases. G6PDd was estimated by Dichloro-phenol-indophenol (DCIP) decolorizing test. The mean age of recruited patients was 28.6 years ranging from 4-70 years. Out of 583 patients tested, 8 (1.37%) were found to be G6PD deficient. G6PDd was more prevalent in *P. vivax* infected patients (2.3%) than the *P. falciparum* (0.4%) (P-value = 0.0401). G6PD deficiency was more prevalent in females (2.06%) than in males (1.23%). The prevalence of G6PDd among the study population is low, but before the long term treatment of primaquine, G6PD deficiency test should be performed to avoid deaths due to primaquine induced acute hemolytic anemia.

### Introduction

The glucose-6-phosphate dehydrogenase (G6PD) deficiency is an erythro-enzymopathological disease in humans, affecting 400 million people worldwide (Howes *et al.*, 2012; Isaac *et al.*, 2013). It is an X-linked recessive, heritable, genetic

disorder, due to mutations in the *G6PD* gene, which cause functional variants with many biochemical and clinical phenotypes. The *G6PD* gene is situated at the telomeric region of the X chromosome (band Xq28) which consists of 13 exons and 12 introns. It

encodes 515 amino acids and a GC-rich (more than 70%) promoter region (Ruwende and Hil, 1981). Approximately 140 mutations or combinations of mutations responsible for this deficiency have been described (Zhao *et al.*, 2010; Manganeli *et al.*, 2013). Glucose-6-phosphate dehydrogenase (G6PD), a metabolic enzyme, plays an active role in survival of erythrocytes. It catalyses the first reaction in the pentose phosphate pathway, providing reducing power to all cells in the form of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate). NADPH enables cells to counterbalance oxidative stress triggered by oxidant agents. In individuals whose G6PD enzyme is deficient, different kinds of haemolysis from mild to severe are seen bound to differences in variants of the disease (Beutler, 1983; Luzzatto 1989).

The disease has been reported nearly from all geographical locations (Tishkoff *et al.*, 2001); however its prevalence is highest in areas where malaria had been endemic for long time like Africa, Central and South America, while in malaria non-endemic regions prevalence is low, suggesting the relationship between G6PD deficiency and malaria (Haworth *et al.*, 1988; WHO, 2003).

One of the significant global public health challenges is controlling malaria, especially in low transmission regions which are seen as prime areas for malaria elimination (WHO, 2011). Therefore, World Health Organization (WHO) strongly recommends the use of primaquine (PQ) for both transmission blocking of *Plasmodium falciparum*, because it kills mature gametocytes, and as anti-relapse treatment against *Plasmodium vivax* by killing liver hypnozoites (White, 2008; Wells *et al.*, 2010). But the use of PQ without knowing the G6PD level may lead to acute

haemolytic anaemia (AHA) in G6PD deficient patients (Howes *et al.*, 2013) which may even cause death. Therefore, screening of G6PD deficiency is very important for malaria patients before treatment with long term PQ.

The report of G6PD deficiency in the malaria endemic areas of India is very few, particularly from West Bengal. Therefore, the present study was conducted to detect the prevalence of G6PD deficiency among the malaria patients (both *P. falciparum* and *P. vivax*) of urban Kolkata, West Bengal who were diagnosed at the Malaria Clinic of Calcutta School of Tropical Medicine.

## **Materials and Methods**

### **Study Population and Collection of Blood Sample**

The present study was conducted from 2012 to 2015 at the Malaria Clinic of Calcutta School of Tropical Medicine, Kolkata. In routine practice febrile patients attending the clinic were examined clinically and thick and thin peripheral blood smear were collected on same glass slide. Both the smears were stained with Giemsa and examined for malarial parasite. Three ml of EDTA blood was collected from known microscopically positive malaria patients for estimation of haemoglobin and qualitative test for screening of G6PD deficiency. Inform consent was obtained from the adult patients and from the guardians of the patients below the age of 14 years before collection of venous blood.

### **Estimation of Haemoglobin**

Haemoglobin (Hb) level of recruited patients was measured by Cyanmethemoglobin method. 20 µl of EDTA blood was drawn with the help of

haemoglobin pipette and transferred into a test tube containing 5 ml of Drabkin's solution (it contains potassium cyanide, potassium ferricyanide, and potassium phosphate). The blood and Drabkin's solution were mixed thoroughly and kept undisturbed for 5 mins. The absorbance of the sample was measured in a photoelectric colorimeter at 540 nm after adjusting the OD at 0 by using Drabkin's solution as blank. The standard Cyanmethemoglobin solution contains 14.8 g of the haemoglobin/100 ml. The corresponding blood haemoglobin in g/100 ml was obtained by multiplying the concentration of the standard solution by dilution factor.

### **Qualitative Test for G6PD Deficiency**

The samples were screened for red cell G6PD deficiency by Dichloro phenol indophenol (DCIP) decolorizing test. For this test HAMOPAK G6PD test kit (Reckon Diagnostic P. Ltd., Vadodara, India) was used and test was performed as per manufacturer instruction. In this test NADPH evolved through the action of G6PD reduces the dye DCIP into a colourless state (DCIPH<sub>2</sub>). The rate and the degree of this decolorization are proportional to the G6PD activity in the RBCs. Phenazone methosulphate (PMS) is used as an electron carrier between NADPH and 2, 6 DCIP in this test. At first 0.5 ml of buffer was added to the Co-enzyme substrate and shaken well to allow complete dissolution. Then 20 µl of whole blood was mixed with pre-cooled lysing reagent and stored in refrigerator (2 - 4°C) for 10 - 15 minutes. After that red cell haemolysate was transferred to the vial containing co-enzyme substrate solution and mixed well. The mixture was immediately overlaid by 1 ml of inert oil and the vial was sealed tightly to make it air tight and incubated at 37°C. The colour of the mixture changes initially from

blue to brownish. In normal G6PD subjects, the decolorization of the mixture will occur within 30 - 60 minutes while it takes 2 - 24 hours for G6PD deficient subjects.

### **Statistical Analysis**

All data were entered and verified using Microsoft Excel software® 2007 and statistical analysis was done by Graph Pad InStat3 (version 3.06) statistical software. Chi-squared or Fisher's exact tests were used to assess significant differences in proportions. All reported P-values are two-sided and were considered statistically significant if <0.05.P value.

### **Results and Discussion**

#### **Demographics of Study Population**

From 2012 to 2015, a total of 583 malaria positive patients were recruited from the Malaria Clinic of Calcutta School of Tropical Medicine, Kolkata. The mean age was 28.67 years (SD: ± 14.62) with a range of 4 to 70 years, distributed as 14.6% was < 15 years, and 85.4% was ≥ 15 years. The male: female ratio was 5.01:1. The demographic data and clinical parameters of the study groups are summarized in Table 1.

#### **Parasitological Parameters**

*P. falciparum* was present in 284 (48.7%) and *P. vivax* in 299 (51.3%) patients. Significant differences were found between *P. falciparum* and *P. vivax* infections for age (P <0.0008), sex (P <0.009), and G6PD deficiency (P <0.0401) (Table 1).

#### **Haematological Parameters**

For all patients, the mean Hb concentration was 12.38 g/dL (95% CI: 12.19-12.58) and was significantly lower in females 11.83

g/dL vs. males 12.84 g/dL (P <0.0001). But no significant difference was observed between the mean haemoglobin concentration of G6PD deficient cases 11.77 g/dL and normal cases 12.42 g/dL (P < 0.1052).

**Prevalence of G6PD Deficiency**

Among the 583 patients screened for G6PD deficiency, 8 (1.37%) cases were found G6PD deficient which includes 2 (25.0%) female and 6 (75.03%) male. The mean age of G6PD deficient cases were 35.0 years (SD: ± 16.71) with range from 16 to 56 years. Mean haemoglobin concentration of G6PD deficient cases was 11.66 g/dL (95% CI: 11.09-12.23). Among the G6PD deficient cases 1 (12.50%) case was *P. falciparum* positive and 7 (87.50%) cases were *P. vivax* positive. The occurrence of G6PD deficient cases was higher in *P. vivax* malaria cases (2.3%) than *P. falciparum* cases (0.4%).

Due to introduction of Artemisinin Combination Therapies (ACTs) the total

number of *P. falciparum* cases decreased from 226 million in 2008 to 201 million in 2014. But the number *P. vivax* infection remained more or less same globally (WHO, 2009; WHO, 2015a). The main problem in controlling *P. vivax* cases is relapse due to presence of dormant hypnozoite stage in liver. PQ is the only known drug effective in hypnozoite (White, 2008) when treated with a longer duration.

As per WHO, *P. vivax* cases are now treated with PQ for 14 days in addition with schizonticidal drug like CQ in areas where CQ is effective. Recently WHO targeted a year wise goal to eliminate malaria from 10 countries by 2020, from 20 countries by 2025 and from 35 countries by 2030 (WHO, 2015b). To achieve this PQ has an important role as gametocidal agent for *P. falciparum* for transmission blocking and to kill hypnozoites of *P. vivax* and *P. ovalae* to prevent relapse (White, 2008; Maude *et al.*, 2012). Use of long term PQ for *P. vivax* may lead to death due to AHA in G6PD deficient patients.

**Table.1** Demographic and Biochemical Parameters of the Study Patients

Patients characteristics	Total	<i>P. falciparum</i>	<i>P. vivax</i>	P-value
Number of patients	583	284	299	-
Age (yrs)				
Mean	28.67	30.74	26.71	0.0008
Range	4-70	4-70	5-68	
SD	± 14.62	± 15.45	± 13.52	
95% CI	27.49-29.86	28.94-32.54	25.18-28.25	
Age category: no (%)				
< 15yrs	85 (14.6)	37 (13.1)	48 (16.1)	0.347
≥ 15 yrs	498 (85.4)	247 (86.9)	251 (83.9)	
Sex ratio (% female)	16.64	20.77	12.71	0.009
Haemoglobin (g/dl)				
Mean	12.38	12.73	11.93	0.0001
Range	7.85-17.04	10.60-14.90	7.85-17.04	
SD	± 1.40	± 1.02	± 1.69	
95% CI	12.19-12.58	12.54-12.92	11.57-12.29	
G6PD deficiency: no (%)	8 (1.37)	1 (0.4)	7 (2.3)	0.0401

**Table.2** Age and Sex Wise Distribution of G6PDd Cases

Characteristics	Study patients (n = 8)
Sex: no (%)	
Male	6 (75.0)
Female	2 (25.0)
Age (yrs)	
Mean	35.0
Range	16-56
SD	± 16.71
95% CI	21.02-48.97
Haemoglobin (g/dl)	
Mean	11.66
Range	10.30-12.4
SD	± 0.68
95% CI	11.09-12.23
Malaria parasites	
<i>P. falciparum</i>	1 (12.50)
<i>P. vivax</i>	7 (87.50)

Prevalence of G6PD deficiency in India varies between 0-28% (Basin and Walter, 2001). In the present study an overall prevalence of G6PDd was 1.37% among the malaria positive patients attending the Malaria Clinic of Calcutta School of Tropical Medicine, Kolkata. Our finding was in accordance to Pao *et al.*, 2005 (Pao *et al.*, 2005) who showed prevalence of 2.0% in Indian general population in his retrospective hospital based study. Jain, 1992 (Jain, 1992) found prevalence of 1.8% in Udaipur Rajasthan in malaria endemic population. We also found that, the deficiency was more prevalent in females (2.06%) than in males (1.23%) which is contradictory to the previous studies by Pao *et al.*, 2005 (2.8% male, 1.1% female) and by Gupte *et al.*, 2005 (27.9% male, 9.7% females) in Vataliya Prajapati communities from Surat. But, Ramadevi *et al.*, 1994 found equal prevalence of G6PD deficiency in males and females and Wang *et al.*, 2009 indicated that sex was not a significant predictor associated with actual G6PD enzyme levels (Wang *et al.*, 2009). This can be explained by the fact that heterozygously deficient women have a mixed population of

erythrocytes, owing to random inactivation of one of the two x-chromosomes, known as lyonization (Davidson, 1963). Due to lyonization some of the heterozygote females are deficient and others are normal (Davidson, 1963).

In conclusion, in the present study G6PD level was estimated among the known malaria patients only and it was a clinic based study. So it does not reflect the overall prevalence among the entire population. But the results showed that 1.37% malaria positive patients were G6PDd. So before giving PQ treatment for longer duration G6PD level should be analyzed.

#### **Authors' Contribution**

AKM designed the study; MC, SG, PS, clinically examined, recruited patients, collected the blood samples; SG, DKB provided the treatment, MC, SG, PS assessed laboratory diagnostics; MC, PS, NB perform the data analysis and interpretation. AKM, PS, SG, NB prepared the manuscript.



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