

Association of Biochemical and Genetic Level Variations of G6PD in Pathogenesis of Plasmodium Falciparum Infected Malaria Cases: A Study from Dimoria Block of Assam

Dipankar Bujarbaruah ¹

Manash Pratim Kalita ²

Saraju Hazarika ³

Abstract: Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common inherited enzymatic disorders and has been implicated in malaria susceptibility and treatment-related haemolysis. However, information on the prevalence of G6PD deficiency and common G6PD gene variants among malaria-endemic tribal populations of Northeast India remains limited. This study investigated the association of biochemical G6PD deficiency and common G6PD genetic variants with Plasmodium falciparum malaria in the Dimoria block of Assam.

Methods: A hospital- and community-based case-control study was conducted involving 153 microscopically confirmed P. falciparum malaria patients (128 uncomplicated and 25 severe malaria cases) and 122 age- and sex-matched healthy controls. G6PD enzyme activity was screened using the fluorescence spot test. Biochemically deficient samples were further analysed for G6PD Orissa (131C>G), G6PD Kerala-Kalyan (949G>A), and G6PD Mediterranean (563C>T) variants by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Statistical analyses were performed using SPSS version 25.0.

Results: Biochemical G6PD deficiency was detected in 17 (11.11%) malaria patients and 8 (6.56%) healthy controls, suggesting a higher prevalence among malaria cases (OR=1.781; 95% CI: 0.741–4.279). Molecular analysis identified only the G6PD Orissa variant in the study population, while the Kerala-Kalyan and Mediterranean variants were absent. The G6PD Orissa variant showed a higher frequency among uncomplicated malaria patients than controls (OR=1.951; 95% CI: 0.351–10.852), although the association did not reach statistical significance. No association was observed with severe malaria.

Conclusions: Biochemical G6PD deficiency and the G6PD Orissa variant were more frequently observed among P. falciparum-infected individuals in this malaria-endemic tribal population of Assam. Although the observed associations were not statistically significant, the findings highlight the importance of G6PD screening before antimalarial therapy and provide baseline genetic epidemiological data for Northeast India. Larger multicentric studies are warranted to validate these observations and to better understand the role of G6PD variants in malaria susceptibility and clinical outcomes.

Keywords: Glucose-6-phosphate dehydrogenase, G6PD deficiency, G6PD Orissa, Plasmodium falciparum, malaria, PCR-RFLP, Assam, Northeast India

^{1,3}Dimoria College²Department of Biotechnology, Gauhati University

World of Medicine: Journal of Biomedical Sciences Vol .3 No.2 (2026)
<https://wom.semanticjournals.org/index.php/biomed>

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency results from structural defect in G6PD enzyme that is imperative for red blood cells survival and oxidative stress response capability. It is an X-linked recessive disorder, and the deficiency affects millions worldwide; and is associated with partial protection against malaria. In India, the prevalence of this deficiency has been reported to vary from 0-27% in different ethnic populations (1,2), with an overall magnitude of 8.5% (3). The *G6PD* gene variants have been linked with varying degrees of severity (4). Mutations in *G6PD* gene results in variants of the expressed proteins rendered with reduced or no functionality, predisposing deficient individuals to haemolysis in presence of certain stressors like drugs that manifest into different clinical syndromes like haemolytic anaemia etc. Therefore understanding the G6PD deficiency pattern and/or the distribution of significant genetic variants of the gene in a geographical population or individuals prior to treatment for malaria especially in malaria endemic areas is of extreme relevance in order to protect patients from haemolytic reactions to anti-malarial drugs.

Northeast India especially specific areas in the state of Assam has been historically endemic *P. falciparum* and *P. vivax* malaria contributing significantly to the overall malaria and malaria-attributable deaths in India (5). The state of Assam is predominantly inhabited by indigenous ethnic tribal communities in specific niches (6, 7); and is predisposed to malaria because of the geographical setup which supports the vector distribution and their livelihood patterns. The present study specifically aimed to study the G6PD patterns and the specific G6PD genetic pattern(s) relevant to Indian context i.e. *G6PD* Orissa, Kerala kalia and Mediterranean variants.

Methods:

Patient enrolment and sample collection

The present study involved clinically proven 153 *P. falciparum* malaria cases (Uncomplicated malaria, UC-M=128; and severe malaria, SM=25] enrolled between 2016-2023 under the supervision of registered medical practitioners and 122 healthy age and sex matched community controls enrolled through health camps with details of any medical history from Dimoria block of Assam which is one of the hyper endemic areas for malaria in Assam and is tribal dominant. Pregnancy cases and children below the age of 18 years and didn't provide informed consent were excluded from the study.

Screening of G6PD enzyme activity and variant analysis

Screening for the G6PD enzyme activity in the blood samples was carried within 12 hours of collection by the Fluorescence Spot Test using commercially available kit and following the manufacturer's instructions. This test was based on the visual evaluation of reduced fluorescence of NADPH under activation with UV light (365nm). Briefly, 10µL of each blood sample was added to 200µL reagent mixture and spotted onto filter paper (Whatman) at time zero. Each sample was incubated at 37°C and spotted again after 5 and 10 minutes of incubation. Fluorescence was observed for the three time points after incubation. Samples which showed no activity after 10 minutes of incubations were considered deficient samples and analysed further for the variant identification.

Assessment of G6PD gene polymorphism in malaria pathogenesis

Many genetic variations have been globally reported for the G6PD gene; but have been prominently studied and reported in Indian context, viz., *G6PD Orissa*, *G6PD Kerala kalia* and *G6PD Mediterranean*. These three polymorphism analysis were studied by Polymerase chain reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method based on the information given in Table 1 given below.

Table 1: List of primers used for PCR amplification and details of restriction digestion based data interpretation for G6PD variant analysis.

Common Variants	Primer sequence 5'→3'	Tm	Restriction enzymes	genotype	Band sizes (bp)
<i>G6PD Orissa</i>	F:5'AGATAGTGGATCAGCAAGCA G3'	60° C	HaeIII	Wild	226
	R:5'GCGAAGTTAGGTTTTGTCA3'			Variant	29,55,130
<i>G6PD Kerala kalia</i>	F:5'CAAGGAGCCCATTCTCTCCCT T3'	60° C	MnlI	Wild	1,6,15,39,54,91
	R:5' TGCCTTGCTGGGCCTCGAAGG3'			Variant	1,15,39,54,97
<i>G6PD Mediterranean</i>	F:5'AGATAGTGGATCAGCAAGCA G3'	60° C	MboII	Wild	277,119,100
	R:5' GCGAAGTTAGGTTTTGTCA3'			Variant	377,119

All statistical analysis was performed using SPSSv25.0 software.

Results

Biochemical screening for G6PD deficiency:

The standard biochemical DPIP method based G6PD screening was performed for all the enrolled malarial patients and healthy controls, the results of which are tabulated in table 2 and 3 below. Altogether 23 cases [8 healthy controls, and 17 malaria cases] were found to be G6PD deficient by biochemical analysis. Overall, the G6PD deficiency is associated with malaria susceptibility in studied cohort.

Table 2. Distribution of G6PD deficient cases in controls and malaria subjects

Subject	N	G6PD Normal	G6PD deficient	p value	ODDS ratio at 95% CI
Control	122	114 [93.44]	08 [6.56]	ref	1.781 (0.741-4.279)
Malaria cases	153	136 [88.88]	17 [11.22]	0.193	

*cases represented as numbers [%age]

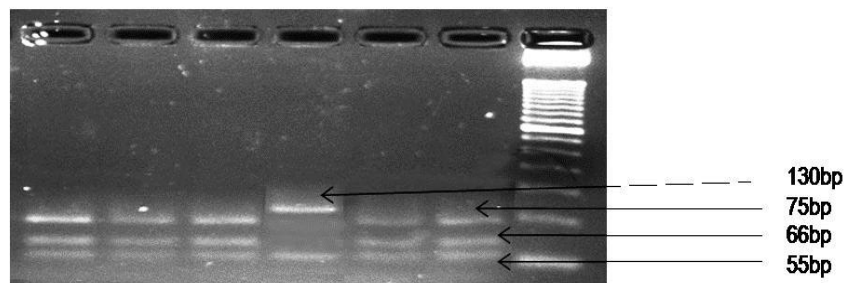
Table 3. Distribution of G6PD deficient cases in controls and malaria sub-cohorts subjects

Subject	N	G6PD Normal	G6PD deficient	p value	ODDS Ratio at 95% CI	P value	ODDS Ratio at 95% CI
Control	122	114 [93.44]	08 [6.56]	ref	ref	NA	NA
UC-M	128	113 [88.28]	15[11.72]	0.159	1.892 (0.772 – 4.637)	Ref	ref
Severe malaria	25	23[92.00]	02[8.00]	0.795	1.239 (0.247 – 6.217)	0.590	0.655 (0.140 -3.062)

*cases represented as numbers [%age]

G6PD variants prevalent among the deficient population:

Three existing G6PD variants were studied by PCR-RFLP for distribution based on literatures available on Indian context viz., G6PD Mediterranean (rs5030868, 563C>T), G6PD Kerala- kalian (rs137852339, 949G>A) and G6PD Orissa (rs78478128, 131C>G). The PCR-RFLP based results indicated the presence of G6PD Orissa variant only in the studied cohorts (Figure 1). The presence of G6PD genetic alterations was observed in a small percentage of control and malaria cases (Table 4), but was found to be associated with increased susceptibility to UC-M compared to controls [OR=1.951(0.351-10.852) at 95%CI, p=0.461) (Table 5).

**Fig 1. Representative agarose gel electrophoresis analysis of PCR-RFLP products for Orissa variant shows the presence of G6PD variant in limited number of subjects.****Table 4. Distribution of G6PD Orissa variant in enrolled controls and malaria cases**

Subject	N	G6PD genotype			P value	ODDS Ratio at 95% CI
		Wild type	Heterozygote	Homozygote		
Control	122	120	02	0	ref	1.611
Malaria cases	153	149	04	0	0.583	(0.290-8.944)

*cases represented as numbers [%age]

Table 5. Distribution of G6PD Orissa variant in enrolled controls and malaria sub-cohorts

Subject	N	G6PD genotype			P value	ODDS Ratio at 95% CI
		Wild type	Heterozygote	Homozygote		
Control	122	120	02	0	Ref	ref
UC-Malaria	127	123	04	0	0.438	1.951 (0.351-10.852)
Severe Malaria	25	25	0	0	0.521	0.828 (0.768-0.891)

Cases represented as N [%age]

Discussion

Malaria caused by the genus *Plasmodium* is a global health issue, the epidemiology of which varies enormously across the globe, and is a leading cause of mortality and morbidity in specific endemic areas

including specific regions of Southeast Asia including specific geographical areas in India. Historically certain areas or blocks in the state of Assam had reported high number of *P falciparum* infected cases including the selected site for this study i.e. the Dimoria block of Assam. *Plasmodium falciparum* malaria is a major cause of morbidity and mortality in northeast India (8, 9). *Plasmodium falciparum* malaria is the most prevalent form in Dimoria block of Kamrup district of Assam, and henceforth, the entire study was focused on the *Plasmodium falciparum* malaria cases from the selected site. As G6PD deficiency and the genetic level variations impact the disease pathogenesis and have clinical relevance, the present study focussed to understand the association of biochemical and genetic level variations of G6PD in the susceptibility and severity of *P falciparum* infected malaria cases.

Glucose-6-phosphate dehydrogenase enzyme acts on the substrate Glucose-6-phosphate in human red cells, and its deficiency inflicts red cell enzymopathy in humans and is one of the inherited disorders. Motulsky et. al., 1966 (10) reported that falciparum malaria confers a selective advantage on G6PD deficiency as well as sickle cell haemoglobin trait. Multiple workers have investigated on the role of G6PD deficiency with respect to malaria, and their scientific data have led to the hypothesis that G6PD deficiency is a polymorphism confers resistance to infection with falciparum malaria (10), generally known as G6PD/ malaria hypothesis. The G6PD enzyme deficiency has been estimated to affecting around 400 million people worldwide (11). In India, G6PD deficiency was first reported in by 12Baxi et al (1963) and the prevalence rate varied from 0–15% in different caste, ethnic and linguistic groups. The population of North-East is unique with different caste and tribal groups, each having their own characteristic physical, cultural and genetic traits. The prevalence of G6PD deficiency has been extensively studied in several population groups, including tribal populations across India (13), with a high frequency been reported from Rabhas (15.8%) and Mikirs (15.6%) in Assam (14). The distribution of G6PD deficiency in the present studied cohort was overall 8.36%. The presence of G6PD deficiency cases were higher in malaria cases (11.11%) compared to healthy controls (6.56%); and was found to be associated with malaria susceptibility (OR=1.781, p=NS).

Genetic variations in G6PD genes are associated with G6PD enzyme deficiency in *P falciparum* malaria (15). On one hand, malaria parasite doesn't grow well in G6PD deficient red cells (16), but on the other hand, G6PD deficient cases are predisposed to antimalarial drugs induced hemolysis. Thirteen biochemically characterized G6PD variants have been reported in the Indian population, with the G6PD Mediterranean mutation (563C→T), G6PD Kerala-Kalyan (949G→A) and G6PD Odisha (131C→G) being the predominant ones (1). Lacunae exist in terms of data on these three genetic variations in northeast Indian in context of malaria cases in ethnically distinct tribal dominant communities in Assam, which was aimed to be addressed through the present study. Presence of G6PD Orissa variant was observed in studied cohorts, and its presence was found to be associated with increased susceptibility to malaria (OR=1.611, p=0.597) especially UC-M compared to controls [OR=1.951, p=0.461].

Conclusion

The presence of Glucose-6-phosphate dehydrogenase deficiency and G6PD Orissa variant is a risk factor for predisposition to *P falciparum* malaria in the studied population.

References:

1. Mohanty et al. 2004, The Indian Journal of Pediatrics, Glucose -6-Phosphate Dehydrogenase Deficiency in India **71**, 525-529. (2004)
2. Devendra et al. 2020, Infection, Genetics and Evolution, Prevalence and spectrums of Mutations causing G6PD deficiency in Indian Population. **86**, 104597. (2020)
3. Kumar et al., Egyptian Journal of Medical Human Genetics, Prevalence of glucose-6-phosphate dehydrogenase deficiency in India: An updated meta-analysis , **17** (3) 295-302 (2016)
4. Rajika L D. et. al. Malaria Journal **14**:93. doi: 10.118 (2015)

5. Dev et al., The Journal of the Association of Physicians of India, Transmission of Malaria and its Control in Northeastern regions of India. **51**, 1073-1076 (2003)
6. Bhasin et al., International Journal of Human Genetics, **6**(3): 233-274 (2006)
7. Bhatia et al., Genetic Atlas of Indian Tribes, National Library of Australia (1987)
8. C. A. Guerra et.al. Lancet 372, 1545 (2008)
9. P.K. mohapatra et al. ICMR Bullt. **28** 21 (1998).
10. Motulsky et. al., American Journal of Human genetics , Population Genetics Studies in Congo. I. Glucose-6-Phosphate Dehydrogenase Defeciency, haemoglobin S, and malaria. **18**(6):514–537 (1966)
11. Luzzotto L, Mehta A. and Meloni T. British Journal of Haematology **91** (2) 511-512 (1995)
12. Baxi et al CABI digital Library (1963)
13. M. B. Mukharjee et. al. Indian Journal of Medical Research **1** (41) 616, (2015)
14. H.M. Bhatiya V. R. Rao Institute of Immunohaematology (ICMR), (1987)
15. D. Mohanty et.al. American . Journal of Haematology; **72**, 150 (2003)
16. J. Roth Jr. S. Schulaman, British Journal of Haematology. **70**, 363 (1988)