

# Assessment of G<sub>6</sub>pd Status in Pre, Post Anti-Malaria Drug Treatment in Malaria Infected and Non-Malaria Infected Individuals on Blood Cell Line Parameters

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**Abstract:** This study was design to determine the effect of G<sub>6</sub>PD status in pre, post anti-malaria drug treatment in *Plasmodium falciparum* malaria infected and non-malaria infected individuals on blood cell line parameters. Malaria infected adult individuals presented with signs and symptoms of malaria infection were used for the study. 202 blood samples were collected twice from each malaria infected individuals; grouped as pre-treatment and post anti-malaria drug treatment. 102 blood samples from apparently healthy individuals were collected as control; both malaria infected subjects and controls were within the age 15-64 years of both sex. 5ml of blood sample was collected and dispensed into di-potassium ethylenediaminetetraacetic acid (K<sub>2</sub>EDTA) vacuitaner bottles for, blood cell parameters which includes absolute platelet count, total white cell count, platelet distribution width, mean platelet volume, relative and absolute white cell differential count were analysed using haematology analyser (Sysmex automated haematology analyser model kx-21n, manufactured by Sysmex co-operation Kobe, Japan), thick blood film was made and stained with Giemsa's staining technique for malaria parasite detection and malaria parasite count, the procedure was described by Monica Cheesbrough. Glucose-6-Phosphate dehydrogenase (G-6-PD) was performed using methaemoglobin reduction method within 6 hours of sample collection. The procedure was as described by Dacie and Lewis. Data obtained was analysed using SPSS version 16. Result of this study showed that, the Mean±SD of MPC, WBC, absolute neutrophil and absolute lymphocyte in pre and post anti-malaria drug treatment was significantly higher in G6PD normal compared to G6PD deficient. Hence, malaria parasite count in G6PD normal was higher compared to G6PD deficient, also immune response to malaria infection was higher in G6PD normal compared to G6PD deficient. G6PD deficiency was associated with significant reduction in the risk of severe malaria. This present study supports the fact that G6PD deficient had genetic resistance to malaria attack compared to G6PD normal.

**Keywords:** malaria parasite, G6PD status and blood cell

## 1. Introduction

The aim of this present was to determine the effect of G<sub>6</sub>PD status in pre, post anti-malaria drug treatment in *Plasmodium falciparum* malaria infected and non-malaria infected (control) individuals on blood cell parameters. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most prevalent enzyme disorder, estimated to affect 400 million people worldwide. Mutations in the glucose-6-phosphate dehydrogenase G6PD gene situated in the long arm of the X chromosome (locus q28), result in different G6PD deficiency variants, producing a wide range of biochemical and clinical phenotypes, which can be further distinguished by differences at the molecular levels (1). G6PD enzyme disorder was discovered in the 1950s when it was found that in some people administration of an anti-malaria drug like premaquine results in hemolytic anaemia. Most of these individuals are otherwise asymptomatic. Similar sort of responses had been reported in cases of a few other drugs, favism and in case of some infections. Since malaria is a disease with high morbidity and high mortality, it therefore has a powerful selective force in human populations, the maintenance of a high frequency of G6PD deficiency despite its deleterious effect of haemolysis is due to its property as a defense against malaria (2, 3). The link between glucose-6-phosphate dehydrogenase G6PD deficiency and malaria is further bolstered by the

observation of the global distribution of glucose-6-phosphate dehydrogenase G6PD deficiency being parallel with that of malaria. In Africa, the prevalence of G6PD deficiency varied, about 28.0% in Nigeria (4). This observation led to the suggestion that G6PD deficiency has protective advantage as a defense against malaria. Some studies have shown that *P. falciparum* parasite densities are lower in G6PD deficient individuals than those with normal (1). A previous study Awah and Uzoegwu, (5) showed that less severe clinical malaria symptoms were observed more in G6PD deficient when compared to G6PD normal. *Plasmodium* infected G6PD deficient cells are thought to impair parasite growth, as they serve as an unstable host for the parasite or behave as a suicidal package being rapidly sequestered in the spleen once it is infected; normal erythrocytes were parasitized by malaria parasites to a much greater extent than G6PD deficient erythrocytes.

## 2. Materials and Methods

### 2.1 Subjects and Study Design

This study was conducted at Federal Medical Centre, Ido-Ekiti, Ekiti State Nigeria; between November 2012 and March 2013. Subjects were *Plasmodium falciparum* malaria infected adult individuals; presented with signs and symptoms of malaria infection. This was confirmed using

malaria rapid kit test and microscopy detection of malaria parasite. Two hundred and two (202) blood samples were collected (5ml) twice from the same malaria infected individuals; grouped as pre-treatment (at presentation) and post anti-malaria drug treatment. One hundred and two (102) blood samples from apparently healthy individuals negative to malaria infection was collected for control; both *Plasmodium falciparum* malaria infected subjects and controls were within the age 15-64 years of both sex. Patient's consent was sort for through an informed consent form; also ethical approval was obtained from the hospital. Structured questionnaire was used to obtained demographic characteristic and other relevant information for the study.

## 2.2 Sample Collection

5ml of blood sample was collected from each subject on the first day of visiting hospital as baseline sample after the patient has been clinically diagnosis for malaria infection, another 5ml of blood sample was collected on the second or third day after taking anti-malaria drugs; blood sample collected was dispensed into di-potassium ethylenediaminetetraacetic acid (K<sub>2</sub>EDTA) vacuitaner bottles.

## 3. Methodology

5ml of blood sample dispensed into di-potassium ethylenediaminetetraacetic acid (K<sub>2</sub>EDTA) vacuitaner bottles were used for blood cell parameters which includes absolute platelet count, total white cell count, platelet distribution width, mean platelet volume, relative and absolute white cell differential count were analysed using haematology analyser (Sysmex automated haematology analyser model kx-21n, manufactured by Sysmex co-operation Kobe, Japan). Thick blood film was made and stained with Giemsa's staining technique for malaria parasite detection and malaria parasite count; observed under microscopy using x100 objective lenses, Malaria parasite counts were estimated by counting malaria parasites against leukocytes (200), and multiply by the patient's own leukocyte count (total white blood cell count); the procedure was described by Monica Cheesbrough, (6). Glucose-6-Phosphate dehydrogenase (G-6-PD) was performed using methaemoglobin reduction method within 6 hours of sample collection. The procedure was as described by Dacie and Lewis (7).

## 4. Statistical Analysis

Data obtained were analyzed for mean and standard deviation; significant test was done by student t-test. Level of significance was considered as <0.05.

## 5. Results

**Table 1:** show the mean  $\pm$  SD of blood cell parameters on G6PD status in pre treatment, post treatment and control. Blood cell parameters include: malaria parasite count (per  $\mu$ l), absolute platelet count (X10<sup>9</sup>), platelet distribution width, mean platelet volume (pL), total white blood cell count (X10<sup>9</sup>), relative neutrophil count(%), relative lymphocyte

count(%), relative monocyte count (X10<sup>9</sup>), absolute lymphocyte count (X10<sup>9</sup>), and absolute monocyte count (X10<sup>9</sup>). The mean  $\pm$  SD of MPC, WBC, absolute neutrophil and absolute lymphocyte 2823.60  $\pm$  296.62, 7.42  $\pm$  1.28 and 4.43  $\pm$  1.18 and 2.68  $\pm$  0.69 respectively in pre treatment G6PD normal were significantly (P<0.05) higher compared to 2092.60  $\pm$  104.51, 4.67  $\pm$  0.61, 2.42  $\pm$  0.56 and 2.01  $\pm$  0.45 respectively in pre treatment G6PD deficient. However, the mean  $\pm$  SD of absolute platelet, PDW, MPV, relative lymphocyte, relative monocyte and eosinophil 166.76  $\pm$  48.22, 13.59  $\pm$  2.48, 9.70  $\pm$  0.76, 36.45  $\pm$  9.02, 3.63  $\pm$  2.23 and 0.86  $\pm$  1.35 respectively in pre treatment G6PD normal were lower compared to 173.74  $\pm$  54.89, 13.63  $\pm$  2.35, 9.96  $\pm$  0.88, 43.26  $\pm$  8.39, 3.78  $\pm$  2.05 and 1.22  $\pm$  1.37 respectively in pre treatment G6PD deficient. The comparison show no significant difference (P>0.05). Hence, the mean  $\pm$  SD of relative neutrophil and absolute monocyte 59.08  $\pm$  9.69 and 0.27  $\pm$  0.19 respectively in pre treatment, G6PD normal were higher compared to mean  $\pm$  SD of relative neutrophil and absolute monocyte 51.80  $\pm$  8.83 and 0.18  $\pm$  0.09 respectively in pre treatment G6PD deficient. Comparison show the significant difference (P>0.05). Moreover, mean  $\pm$  SD of MPC, WBC, absolute neutrophil, lymphocyte and monocyte 2455.80  $\pm$  555.20, 6.59  $\pm$  1.73, 3.61  $\pm$  1.20, 2.84  $\pm$  0.82 and 0.12  $\pm$  0.13 respectively in post treatment G6PD normal were significantly (P<0.05) higher compared to 1892.40  $\pm$  91.91, 3.79  $\pm$  0.59, 1.86  $\pm$  0.57, 1.84  $\pm$  0.34 and 0.07  $\pm$  0.06 in post treatment G6PD deficient. However, the mean  $\pm$  SD of platelet, PDW, MPV, relative lymphocyte and eosinophil 178.47  $\pm$  56.91, 13.72  $\pm$  2.53, 9.54  $\pm$  0.64, 43.36  $\pm$  7.85 and 0.45  $\pm$  0.84 respectively in post treatment G6PD normal were lower compared to 183.35  $\pm$  59.16, 13.77  $\pm$  2.30, 9.79  $\pm$  0.66, 48.76  $\pm$  6.99 and 0.61  $\pm$  0.99 in post treatment G6PD deficient. The comparison show no significant difference (P>0.05); hence, mean  $\pm$  SD of relative neutrophil and monocyte 54.38  $\pm$  8.45 and 1.82  $\pm$  1.52 respectively in post treatment G6PD normal were higher compared to mean  $\pm$  SD of relative neutrophil and monocyte 48.85  $\pm$  7.57 and 1.80  $\pm$  1.52 respectively in post treatment G6PD deficient. The comparison show no significant difference (P<0.05). Furthermore, mean  $\pm$  SD of MPV 9.53  $\pm$  0.23 in control G6PD normal was significantly (P<0.05) higher compared to 9.41  $\pm$  0.36 in control G6PD deficient. Also the mean  $\pm$  SD of platelet, WBC, relative neutrophil, monocyte, absolute neutrophil and absolute monocyte 285.34  $\pm$  47.97, 4.42  $\pm$  0.34, 57.08  $\pm$  4.78, 0.91  $\pm$  1.09, 2.53  $\pm$  0.29 and 0.04  $\pm$  0.05 respectively in control G6PD normal were lower compared to 288.18  $\pm$  48.65, 4.48  $\pm$  0.29, 59.00  $\pm$  3.13, 1.09  $\pm$  1.04, 2.66  $\pm$  0.21 and 0.05  $\pm$  0.05 in control G6PD deficient. The comparison show no significant difference (P>0.05) however, the mean  $\pm$  SD of PDW, relative lymphocyte, eosinophil and absolute lymphocyte 12.05  $\pm$  1.76, 41.78  $\pm$  4.55, 0.33  $\pm$  0.70 and 1.84  $\pm$  0.23 respectively in control G6PD normal were higher compared to 11.70  $\pm$  1.69, 39.72  $\pm$  2.90, 0.18  $\pm$  0.40 and 1.78  $\pm$  0.16 in control G6PD deficient. The comparison show no significant difference (P>0.05)

## 6. Discussion

Out of 202 *falciparum malaria* malaria patients used in this study, 148 were G6PD normal and 54 were G6PD deficient, among the control group, 91 were G6PD normal and 11

were G6PD deficient. Prevalence of G6PD status in this present study was similar to Francis et al 2012 reported of the four (400) individuals screened for this G6PD deficiency, 347 (86.75%) had normal G6PD levels and 53 (13.25%) were G6PD deficient of which 36 (9.0%) were heterozygous and 17 (4.25%) were homozygous. The high frequency of G6PD deficiency in the study population corroborates the role malaria play in the distribution of G6PD genes in most malaria endemic areas in the world (8). G6PD deficiency was associated with significant reduction in the risk of severe malaria for both G6PD deficient females and males in accordance with the reports of Uzoegwu and Awah (5). G6PD deficient parasitized erythrocytes could therefore have been phagocytosised earlier thereby destroying the malaria parasite. Blood cell parameters on G6PD status in patient infected with *plasmodium falciparum* showed that malaria parasite count was significantly higher in G6PD normal compared to G6PD deficient in pre treatment and post treatment. This present study supports the fact that G6PD deficient had genetic resistance to malaria attack compared to G6PD normal. This finding was supported by Francis and Pete (9), stated that, G6PD non-deficient subjects suffered more malaria attack, had significantly higher parasite densities and higher percentage parasitaemia than the G6PD deficient dominant homozygotes. However, there was significant decrease in malaria parasite count mean value of both G6PD normal and G6PD deficient in post treatment, this observation in this study was due to the effect of anti-malaria used during treatment. Mean absolute platelet count, mean cell volume (MPV), platelet distribution width (PWD) in G6PD normal was observed lower compared to G6PD deficient in both pre treatment and post treatment. Thrombocytopenia was observed higher in G6PD normal compared to G6PD deficient, this observation was due to susceptibility of G6PD normal to malaria attack while low thrombocytopenia in G6PD deficient was due to genetic resistance of G6PD deficient to malaria attack. This finding was similar to Francis and Pete (9), reported that there was low platelet count in G6PD non-deficient subjects compared to G6PD deficient is consistent with the finding that platelets could form 'clumps' with *Plasmodium*-infected erythrocytes, hence thrombocytopenia may be helpful as a sensitive but not specific marker of active infection (10, 11). However, low amount of platelets may not only be a marker of parasite burden but may be protective against severe disease. The mean value of mean platelet volume was observed decrease in post treatment compared to pre-treatment compared while mean value of absolute platelet count and platelet distribution width were observed increase in post treatment although there is no significant difference. This is due to effect of anti malaria drug which improve the platelet count during treatment. Immune response to malaria attack in G6PD normal was observed and significantly higher compared to G6PD deficient. Total white blood cell, absolute and relative neutrophil, relative eosinophil, lymphocyte and monocyte were observed higher in G6PD normal compared to G6PD deficient in pre treatment and post treatment while absolute lymphocyte, monocyte and eosinophil were observed lower in G6PD normal compared to G6PD deficient in pre treatment and post treatment. Similar to this present study Francis and Pete 2006 report that, G6PD non-deficient subjects had significantly higher

WBC and granulocyte counts. Contrary to this study, they report that lymphocyte counts were statistically no significantly higher in G6PD non-deficient subjects than G6PD deficient homozygotes. In this present study, there was slight lymphocytosis observed in post treatment. However, immune response to malaria infection was observed to decrease generally in post treatment, this was due to the recovery of the patient after treatment. Blood cell parameters on G6PD status in malaria negative subject (control) were within the normal range. There is no significant difference in most of the blood cell parameters in control subjects since; they are negative to malaria infection. The values in post treatment and control subject was relatively closer, this present study showed that there was recovery from malaria infection after the anti-malaria treatment.

## 7. Conclusion

Malaria parasite count in G6PD normal was higher compared to G6PD deficient, also immune response to malaria infection was higher in G6PD normal compared to G6PD deficient. Immune response to malaria infection was observed to decrease generally in post anti-malaria drug treatment; this was due to the recovery of the patient after treatment. Susceptibility of G6PD normal to malaria attack may cause thrombocytopenia. G6PD deficiency was associated with significant reduction in the risk of severe malaria. This present study supports the fact that G6PD deficient had genetic resistance to malaria attack compared to G6PD normal.

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**Table 1:** Means  $\pm$  SD of Blood Cell Parameters on G<sub>6</sub>pd Status in Pre Treatment, Post-Antimalaria Drug Treatment in Malaria Infected Subjects and Control

Groups Parameters	Pre Treatment		P value	Post Treatment		p. value	Control		p. value
	Normal (N=148)	Deficient (N=54)		Normal (N=148)	Deficient (N=54)		Normal (N=91)	Deficient (N=11)	
MPC $\mu$ /l	2823.60 $\pm$ 296.62	2092.60 $\pm$ 104.51	0.00*	2455.80 $\pm$ 555.20	1892.40 $\pm$ 91.96	0.00*	-	-	-
PLATELET X10 <sup>9</sup> /L	166.76 $\pm$ 48.22	173.74 $\pm$ 54.89	0.06	178.47 $\pm$ 56.91	183.35 $\pm$ 59.16	0.25	285.34 $\pm$ 47.97	288.18 $\pm$ 48.65	0.89
PDW Fl	13.59 $\pm$ 2.48	13.63 $\pm$ 2.35	0.43	13.72 $\pm$ 2.53	13.77 $\pm$ 2.30	0.39	12.05 $\pm$ 1.76	11.70 $\pm$ 1.69	0.98
MPV Pl	9.70 $\pm$ 0.76	9.96 $\pm$ 0.88	0.15	9.54 $\pm$ 0.64	9.79 $\pm$ 0.66	0.51	9.53 $\pm$ 0.23	9.41 $\pm$ 0.36	0.01*
WBC X10 <sup>9</sup> /L	7.42 $\pm$ 1.28	4.67 $\pm$ 0.61	0.00*	6.59 $\pm$ 1.73	3.79 $\pm$ 0.59	0.00*	4.42 $\pm$ 0.34	4.48 $\pm$ 0.29	0.22
NEUTROPHIL %	59.08 $\pm$ 9.69	51.80 $\pm$ 8.83	0.13	54.38 $\pm$ 8.45	48.85 $\pm$ 7.57	0.11	57.08 $\pm$ 4.78	59.01 $\pm$ 3.13	0.18
LYMPHOCYTE %	36.45 $\pm$ 9.02	43.26 $\pm$ 8.39	0.14	43.36 $\pm$ 7.85	48.76 $\pm$ 6.99	0.10	41.78 $\pm$ 4.55	39.72 $\pm$ 2.90	0.17
MONOCYTE %	3.63 $\pm$ 2.23	3.78 $\pm$ 2.05	0.52	1.82 $\pm$ 1.52	1.80 $\pm$ 1.52	0.54	0.91 $\pm$ 1.05	1.09 $\pm$ 1.04	0.62
EOSINOPIL %	0.86 $\pm$ 1.35	1.22 $\pm$ 1.37	0.09	0.45 $\pm$ 0.84	0.61 $\pm$ 0.99	0.07	0.33 $\pm$ 0.70	0.18 $\pm$ 0.40	0.16
NEUTROPHIL X10 <sup>9</sup> /L	4.43 $\pm$ 1.18	2.42 $\pm$ 0.56	0.00*	3.61 $\pm$ 1.20	1.86 $\pm$ 40.57	0.00*	2.53 $\pm$ 0.29	2.66 $\pm$ 0.21	0.06
LYMPHOCYTE X10 <sup>9</sup> /L	2.68 $\pm$ 0.69	2.10 $\pm$ 0.45	0.00*	2.84 $\pm$ 0.82	1.84 $\pm$ 0.34	0.00*	1.84 $\pm$ 0.23	1.78 $\pm$ 0.16	0.23
MONOCYTE X10 <sup>9</sup> /L	0.27 $\pm$ 0.19	0.18 $\pm$ 0.09	0.09	0.12 $\pm$ 0.13	0.07 $\pm$ 0.06	0.02*	0.04 $\pm$ 0.05	0.05 $\pm$ 0.05	0.98

P<0.05 Significance, P>0.05 no Significant.