Glucose-6-phosphate dehydrogenase status and severity of malarial anaemia in Nigerian children

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Abstract

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) deficiency (Gd–) contributes to morbidity and mortality in sub-Saharan Africa but recent data on the interaction between Gd– and malaria among children is scarce. We hypothesised that, being a haemolytic factor, Gd– makes severe malarial anaemia (SMA) more common and even more severe.

Methodology: We selected 930 children aged 0.5–12 years attending a reference hospital with microscopically proven falciparum malaria. G6PD and haemoglobin were typed by the fluorescent spot test and electrophoresis, respectively. Molecular typing by PCR and restriction enzyme digestion was also performed on 15% of randomly selected samples. Haematocrit (PCV) values, haemoglobin type, blood group, presence of sickle cell trait (HbAS), and parasite counts were compared between G6PD-normal and deficient children.

Results: Prevalence of Gd– was 16.4% and 8.1% among boys and girls with malaria, respectively. Mean PCV was 22.8% in deficient children compared with 21.0% in normal children (p=0.041). In boys, 2.7% of Gd– had PCV ≤10%, as compared to 13.6% in Gd+ (p = 0.005). Similarly, 21.3% of Gd– had PCV ≤15% compared with 39.4% in Gd+ (p=0.003). No such difference was found among girls. Overall, HbAS was typed in 7.6% and was more common in Gd– (13.0%) than in Gd+ (6.8%), but the difference was not statistically significant (p=0.058). The mean parasite counts were significantly lower in Gd– (15477.5/μl) than in Gd+ (19784.4/μl; p=0.013), and it was independent from HbAS.

Conclusion: Gd– males but not females were significantly less likely to develop severe malarial anaemia.

Key words: glucose-6-phosphate dehydrogenase deficiency; malarial anaemia; haemolytic anaemia,


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Introduction

The magnitude of morbidity from malaria among children living in sub-Saharan Africa continues to be of public health concern, in spite of efforts at its control. The co-existence of malaria and G6PD deficiency in the same sub-region constitute an even greater global health problem and threat to the life of children [1,2]. Malaria is the most common cause of severe anaemia among Nigerian children and severe malarial anaemia accounted for about three-quarter of all cases of malaria morbidity as well as 9% of all admissions in a typical paediatric emergency ward [3].

Anaemia due to malaria has been described as normocytic with intravascular haemolysis, extravascular clearance of parasitized red blood cells, and bone marrow dysfunction the general features [4,5]. Red cell destruction and decreased red cell production are the major mechanisms that explain the development of anaemia during acute malaria episodes [6]. On the other hand, acute haemolytic anaemia is the most frequent clinical manifestation of G6PD deficiency (Gd–) [7,8]. The haemolysis is precipitated most commonly by infections such as malaria but can also occur after ingestion of drugs, including some antimalarials and foods that contain oxidants or in certain metabolic conditions such as diabetic ketoacidosis [9].

The fact that G6PD-deficient alleles have reached polymorphic frequencies in tropical and subtropical environments where malaria is endemic and the total absence of polymorphic frequencies of the alleles in areas of low endemicity suggest a relationship between G6PD deficiency and malaria morbidity [10]. Therefore, very high malaria-endemic areas such as Nigeria may have a different pattern of association between G6PD status and malarial anaemia than less endemic areas as both factors can
independently cause or worsen anaemia in children by potentiating red blood cell haemolysis. The coexistence of G6PD deficiency and malaria infection in an individual may increase the risk of developing severe anaemia.

In Nigeria, G6PD deficiency occurs in 24% of boys and 5% of girls [11]. It is also known to be a significant cause of anaemia in children, especially neonates [7]. The interactions between Gd− and malaria have been variously discussed in the literature [12-15]. Although most published data indicate that Gd− protects against severe malaria, they come from areas where malaria is less endemic and Gd− less common than they are in Nigeria [16]. There is a dearth of data on the effects of the coexistence of these two known haemolytic factors in children who suffer from acute *P. falciparum* malarial infection. We therefore examined the relationship between Gd− and severity of malarial anaemia in Nigerian children. It was hypothesised that, being a haemolytic factor, host Gd− makes malarial anaemia (SMA) more common and more severe, independent of the sickle cell trait (HbAS).

**Methodology**

**Study design and setting**

This was a cross-sectional study involving consecutive children with microscopically proven *falciparum* malaria. The study was conducted at the children’s emergency ward and out-patient clinics of the University College Hospital (UCH), Ibadan. The hospital is located in the Ibadan North Local Government Area. Ibadan is an urban city in the southwest of Nigeria, a region of malaria hyperendemicity. According to the 2006 National Census, Ibadan has an estimated population of 2.5 million, an annual growth rate of 2.8%, and the majority of the inhabitants are Yoruba people. The UCH was established to serve the people of Ibadan city and other parts of Nigeria. All children aged six months to 12 years with severe malaria are first admitted to the children’s emergency ward, which has a laboratory where simple ancillary investigations including microscopy and haematocrit are performed. Every child with fever routinely gets screened for malaria and anaemia.

**Sample size and power calculation**

Using the prevalence of 24% (in the general population) as the hypothesized value and an alternative hypothesized prevalence of 16% among malaria patients, a sample size of 930 children would give an estimated power of 90% at a 95% level of confidence (two-sided). These estimates were obtained using the module for one-sample comparison of proportion to hypothesized value in Stata/IC 11.0 software (Stata Corporation, College Station, USA).

**Sampling procedure**

Children who presented with fever at the children’s emergency ward and children’s out-patient clinics consecutively had peripheral blood film examination for *P. falciparum*. Those who had malaria parasitaemia were recruited provided there was no other obvious cause for fever, such as respiratory infections. Written informed consent was sought from the caregivers. To minimise selection bias at the point of entry, there were no known distinguishing features between malaria positive and negative children until G6PD screening was done. Patients who were later found to have haemoglobin genotype SS or SC determined by haemoglobin electrophoresis on cellulose acetate using whole blood samples [17] and those who had blood transfusion within three months before enrolment were excluded from the study.

**Data collection procedure**

Data were obtained from the caregivers and the children, and a physical examination including general appearance, chest, cardiovascular, abdominal and central nervous system was performed on each patient. All findings including the results of the G6PD test, PCV, parasite counts, blood groups, and haemoglobin types were recorded by the investigators into a standardized structured form. Severe malaria was defined according to World Health Organization (WHO) criteria [18].

**Laboratory procedures**

Thick and thin films were made on the same slide for each patient. The thin film was fixed with methanol immediately and the slide was allowed to dry, then flooded with Giemsa stain already diluted one in 10 with phosphate buffer pH 6.8. The stain was washed off with distilled water, after allowing it to stay for about 10 minutes, and air-dried. The stained films were then examined microscopically for malaria parasites. The parasites were counted against 200 white blood cells (WBC) and parasite density was calculated for each patient based on assumed total WBC of 8000/µl of blood.
Packed cell volume (PCV) was determined using capillary methods and spinning in a micro-
haematocrit centrifuge (Hawksley, Sussex, United
Kingdom) at 11,000 g for 5 minutes at room
temperature.

Blood Grouping: ABO blood groups were
determined using the agglutination tile method.
G6PD status was determined by fluorescent spot test
according to the method of Beutler and Mitchell [19].
This test is based on the principle that NADPH
generated in red cells in the presence of G6PD
fluoresces under long wavelength ultraviolet (UV)
light. The drawback of this method is that it does not
distinguish between heterozygote and homozygote
normal females.

Quality control

A parallel G6PD screening test of about 60% of
the patients in each batch was carried out in the
paediatric research laboratory. This laboratory has
been running G6PD screening for over 15 years.
Some of the children with severe anaemia (PCV <
15%) had repeated G6PD screening three months
after blood transfusion. There was over 98%
agreement rate in the results obtained (kappa = 0.99).
In addition, about 15% of the blood samples were
randomly selected and subjected to molecular typing
by PCR and restriction enzyme digestion and the
concordance rate between molecular and biochemical
analysis was 99.20%.

Data management and analysis

Data were entered, rechecked and analysed using
SPSS 15.0 for Windows (SPSS Inc., Chicago, USA).
Z-scores for weight-for-height (WHZ) were
calculated using the National Center for Health and
Statistics (NCHS) reference charts. Stratified analysis
of the various manifestations of malaria infection by
gender was conducted to control for confounding.
Categorical variables were compared using either the
uncorrected chi square test or Fisher’s exact test
while continuous variables were analyzed using the
Student t test or analysis of variance (ANOVA). Data
not normally distributed was compared using the
Mann-Whitney U test. Estimates of continuous
variables were expressed as mean (± SD) while
categorical variables were in proportions. Statistical
significance level was set at p < 0.05.

Ethical approval

Ethical approval was obtained from the
University of Ibadan/University College Hospital
ethical review committee for this study. Written
informed consent was obtained from children’s
caregivers.

Results

A total of 930 children (458 male and 472 female
[M:F = 1:1.03]) with symptomatic *P. falciparum*
malaria were screened for G6PD deficiency. The age
distribution of participants showed that the majority
of the children with malaria was under five years.
The modal age group was 12-23 months and the
median was 35 months. Infants constituted 11.8%
while children under the age of five years constituted
77.3% of all the study patients. There was no
significant difference between male and female
representation (p = 0.672). Other demographic
characteristics are given in Table 1. Overall, 113
(12.2%) were G6PD deficient, giving a prevalence
rate of 16.4% and 8.1% among male and female
malaria patients, respectively. The prevalence of
G6PD deficiency was significantly higher in males
than in females (p < 0.001). The mean (± SD)
weight-for-age z-scores were -1.10 (± 0.19) and
-1.27 (± 0.07) among the G6PD normal and deficient
patients, respectively (p = 0.821).

The comparisons of the PCV, haemoglobin (Hb)
types, and blood group distributions between the
G6PD normal and deficient patients are shown in
Table 2. PCV of patients ranged from 6% to 45%
(mean PCV = 21.1%). The mean PCV of G6PD
normal patients (21.0%) was slightly but significantly
lower than in the deficient patients (22.8%; p =
0.041). Ninety-eight (86.7%) patients had Hb type A
among the G6PD-deficient group compared with 722
(88.4%) patients in the G6PD-normal group (p =
0.611). The proportion of G6PD-deficient patients
with blood groups O, A, B and AB were 57.5%,
23.0%, 15.9% and 3.6%, respectively. This pattern of
distribution did not significantly differ from 58.9%,
18.7%, 19.2%, and 3.2% for blood groups O, A, B
and AB, respectively among the G6PD
normal patients.

Table 3 shows comparisons of parasite counts
between G6PD-deficient and G6PD-normal groups,
stratified by gender. In the male category, the mean
of parasite counts in males with G6PD deficiency
(15397.0/µl, range = 340 - 860,000) was significantly
lower than in those with normal G6PD (24662.8/µl,
range = 112 –1,824,400); p = 0.041. Conversely, in
the female category, though the G6PD-deficient

Table 1. Characteristics of the study participants

<table>
<thead>
<tr>
<th>Gender</th>
<th>G6PD Status</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient (113)</td>
<td>Normal (817)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>75 (16.4%)</td>
<td>383 (83.6%)</td>
<td>458 (100.0%)</td>
</tr>
<tr>
<td>Female</td>
<td>38 (8.1%)</td>
<td>434 (91.9%)</td>
<td>472 (100.0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>G6PD Status</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient (113)</td>
<td>Normal (817)</td>
<td></td>
</tr>
<tr>
<td>Min - Max</td>
<td>6-144</td>
<td>6-138</td>
<td>6-144</td>
</tr>
<tr>
<td>Median</td>
<td>40.0</td>
<td>34.0</td>
<td>35.0</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Weight-for-age</th>
<th>G6PD Status</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient (113)</td>
<td>Normal (817)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-1.10</td>
<td>-1.27</td>
<td>-1.25</td>
</tr>
<tr>
<td>SD</td>
<td>0.19</td>
<td>0.07</td>
<td>0.07</td>
</tr>
</tbody>
</table>

NB: *Mann-Whitney U test (M-W U), 'Chi-square test (χ²) SD – Standard Deviation

Table 2. Haemoglobin types and blood groups of G6PD normal and deficient patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>G6PD Status</th>
<th>All patients</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient (113)</td>
<td>Normal (817)</td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min – Max</td>
<td>340 – 860000</td>
<td>112 – 1824400</td>
<td>102 – 1824400</td>
</tr>
<tr>
<td>Mean</td>
<td>15477.5</td>
<td>19784.4</td>
<td>15956.7</td>
</tr>
<tr>
<td>Median</td>
<td>55987</td>
<td>74208</td>
<td>58250</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min – Max</td>
<td>340-840000</td>
<td>236-1824000</td>
<td>236-1824000</td>
</tr>
<tr>
<td>Mean</td>
<td>15397.0</td>
<td>24662.8</td>
<td>16658.9</td>
</tr>
<tr>
<td>Median</td>
<td>12640</td>
<td>23553</td>
<td>19600</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min – Max</td>
<td>960-860000</td>
<td>102-969882</td>
<td>102-969882</td>
</tr>
<tr>
<td>Mean</td>
<td>12840.4</td>
<td>15548.6</td>
<td>15304.9</td>
</tr>
<tr>
<td>Median</td>
<td>12800.0</td>
<td>13931.0</td>
<td>12800.0</td>
</tr>
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</table>

Table 3. Malarial parasite counts of G6PD normal and deficient patients

<table>
<thead>
<tr>
<th>G6PD Status</th>
<th>All patients</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient (113)</td>
<td>Normal (817)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>22.8</td>
<td>21.0</td>
</tr>
<tr>
<td>SD</td>
<td>7.9</td>
<td>8.9</td>
</tr>
<tr>
<td>Haemoglobin type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>98 (86.7%)</td>
<td>722 (88.4%)</td>
</tr>
<tr>
<td>AS</td>
<td>15 (13.3%)</td>
<td>95 (11.6%)</td>
</tr>
<tr>
<td>Blood group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>65 (57.5%)</td>
<td>481 (58.9%)</td>
</tr>
<tr>
<td>A</td>
<td>26 (23.0%)</td>
<td>153 (18.7%)</td>
</tr>
<tr>
<td>B</td>
<td>18 (15.9%)</td>
<td>157 (19.2%)</td>
</tr>
<tr>
<td>AB</td>
<td>4 (3.6%)</td>
<td>26 (3.2%)</td>
</tr>
</tbody>
</table>

NB: Min – Minimum, Max – Maximum, 'Mann-Whitney U test
group had lower mean parasite counts (12840.4/µl, range = 960 - 860000), there was no significant difference compared to those with normal G6PD (15548.6/µl, range = 102-969882); p = 0.419.

Defining the degree of anaemia as PCV less or equal to 10%, 15%, 20%, 25% and 30% in order of decreasing severity (Table 4), in the male category, the proportions of patients whose PCVs were less or equal to 10% and 15% among the G6PD-deficient group were significantly lower than their counterparts in the G6PD-normal group (p < 0.05). Also, patients who were G6PD-deficient had significantly lower chances for severe malarial anaemia, at PCV of equal to or less than 10% (OR = 0.17, 95% CI = 0.04, 0.73 and 15% (OR = 0.42, 95% CI = 0.23, 0.75). There was no significant association between anaemia defined as PCV cut-offs equal to or above 20% and G6PD status among the male patients. Unlike in males, the data from the female group showed no significant association between G6PD status and any of the various degrees of anaemia, defined by PCV cut-offs of 10%, 15%, 20%, 25% and 30% (Table 5).

Of the 930 study patients, there were six deaths giving an overall case fatality of 0.7%. Whereas no deaths occurred among the G6PD-deficient patients in this study, all the deceased patients were G6PD normal. Visual and hearing impairment was documented at the time of discharge in one patient with cerebral malaria.

**Discussion**

Contrary to our hypothesis, this study showed that G6PD deficiency, a haemolytic factor among Nigerian children, neither makes malarial anaemia (SMA) more common nor more severe. The G6-males were significantly less likely to develop severe malarial anaemia. The overall prevalence of G6PD deficiency among children (males and females) with malaria in this study was 12.2%. This value was lower than the frequency of 20% reported among 30 children with a mean age of 3.6 years who were brought to hospital with convulsions and *falciparum* malaria infection in Ibadan [20]. Similarly, the respective prevalence rates of 16.4% and 8.1% among male and female malaria patients in the present study are at variance with the respective figures of 23.9% and 4.6% among males and females reported recently by Ademowo and Falusi [11]. The prevalence of G6PD deficiency among males is four times higher than in females in the general population. It was therefore not unexpected that in the population of children with malaria, the frequency of G6PD deficiency among males would be significantly higher than that of females as obtained in the present study. While this study was carried out among children aged 6 months to 12 years (76% were under five years), many of the previous prevalence studies [10,21,22] were performed among mixed populations of children and adults [11,20,21]. This factor may explain the differences in the prevalence rates.

In *P. falciparum* malaria endemic regions, anaemia poses a great danger to child survival. Most children with severe malarial anaemia often require blood transfusions [22,23]. Apart from the many forms of blood transfusion reactions that may occur, the risk of blood human immunodeficiency virus and hepatitis virus transmission increase with successive blood transfusion Furthermore, children who are G6PD deficient are thought to have potentially higher risk of blood human immuno-deficiency virus infection. It would be expected that malaria patients who are G6PD deficient would have a relatively lower mean haematocrit level. However, using the different levels of haematocrit cut-offs to examine the effects of G6PD status on anaemia, the results from this study showed that at haematocrit cut-offs of ≤ 10% and ≤ 15%, the G6PD-deficient male patients were 5.9 and 2.4 times respectively less likely to have anaemia. In clinical terms, this suggests that the G6PD-deficient children are protected against severe
malarial anaemia when defined as haematocrits of 15% and below.

The mean parasite count of the males was significantly lower than that in normal children but no difference was found among female children in this study. These results suggest that G6PD deficiency may have protected the males against high parasitaemia and not females. The finding in the male group agrees with the findings of the study by Guindó et al. [24] who showed that the uniform state of G6PD deficiency in hemizygous male children conferred a significant protection against severe, life-threatening malaria, and that it may have likewise protected homozygous female children. No such protection was evident from the mosaic state of G6PD deficiency in heterozygous females. The lack of difference in the parasite rate between G6PD deficient and normal female malarial patients was previously reported [24]. On the contrary, evidence from another series of studies in Nigeria [25], suggests that the G6PD deficient heterozygous females were relatively protected against *falciparum* malaria, but not the more severely deficient hemizygous males and homozygous females. Studies have also demonstrated differential levels of parasitization in G6PD deficient and non-deficient red cells in the same individual; the parasite rates in the G6PD-normal erythrocytes were six to 81 times greater than the rate of parasitization in the G6PD-deficient erythrocytes in the same individuals [25].

One of the main mechanisms by which anaemia develops in malaria patients is erythrocyte destruction in the reticuloendothelial system [26]. As this study found that G6PD-deficient patients had significantly lower parasite counts than normal patients, it is plausible that there were relatively fewer parasitized red cells for destruction in the G6PD-deficient group. This may explain the lower frequency of children with severe forms of anaemia. Also, the findings of relative protection against occurrence of severe anaemia supported previous reports on the poor growth of malaria parasites in G6PD-deficient red cells in *in-vitro* studies [27,28].

The drawback of the G6PD deficiency screening test (fluorescent spot) used in this study is that it does not distinguish between heterozygote and homozygote normal. This disadvantage made it difficult to further explore the degree of G6PD deficiency; it is therefore not unlikely that some of the patients classified as having normal G6PD may in fact have had some degree of deficiency not detected by the fluorescent spot method. No biochemical test was done to detect ingestion of oxidant agents such as drugs before the patients presented in the hospital. This factor also made it difficult to make clear the extent to which pre-hospital medication may have contributed to the development of anaemia reported in this study. Though the lack of difference in the mean age of G6PD deficient and normal patients suggests that the two groups are comparable in terms of age, the effect of age on malaria morbidity among both patient groups remains undetermined in this study.

### Acknowledgments

The authors appreciate the immense laboratory work and assistance rendered by Mr. Nath Afolabi and other scientists in the Department of Paediatrics, University College Hospital, Ibadan. We also appreciate the cooperation and assistance of all the staff of the Department of Paediatrics, University College Hospital, Ibadan, especially the resident doctors and nursing staff.

### References


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**Table 5. Malarial anaemia and G6PD status among the female patients**

<table>
<thead>
<tr>
<th>PCV</th>
<th>G6PD Status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=38</td>
<td>N=434</td>
</tr>
<tr>
<td>≤ 10%</td>
<td>4 (10.5)</td>
<td>49 (10.4)</td>
</tr>
<tr>
<td>≤ 15%</td>
<td>13 (34.2)</td>
<td>165 (35.0)</td>
</tr>
<tr>
<td>≤ 20%</td>
<td>19 (50.0)</td>
<td>241 (51.1)</td>
</tr>
<tr>
<td>≤ 25%</td>
<td>23 (60.5)</td>
<td>325 (68.9)</td>
</tr>
<tr>
<td>≤ 30%</td>
<td>31 (81.6)</td>
<td>383 (81.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.975</td>
<td>1.02</td>
<td>0.35, 3.0</td>
</tr>
<tr>
<td>0.942</td>
<td>0.97</td>
<td>0.48, 1.96</td>
</tr>
<tr>
<td>0.891</td>
<td>0.95</td>
<td>0.49, 1.85</td>
</tr>
<tr>
<td>0.247</td>
<td>0.67</td>
<td>0.34, 1.33</td>
</tr>
<tr>
<td>0.943</td>
<td>1.03</td>
<td>0.44, 2.43</td>
</tr>
</tbody>
</table>

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**Note:** The table displays the distribution of malarial anaemia and G6PD status among the female patients, showing the frequency of anaemia in different PCV levels for G6PD deficient and normal groups, along with the calculated odds ratios and 95% confidence intervals.

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**Conflict of interests:** No conflict of interests is declared.