The Impact of Human Genetic Factors (G6pd and Type of Hemoglobin) on the Course of Uncomplicated Malaria Infection in Children Aged from 2 to 10 Years Living in the Banfora Health District in Burkina Faso

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Authors’ contributions
This work was carried out in collaboration among all authors. Authors SS, IS, YI, AD, AST, ABT, BSS and MMD designed the study, wrote the protocol, performed the study analysis and wrote the first draft of the manuscript. Authors SSS, AD, SAC, AB, DK ensure the participant follow up and collected field data. All authors read and approved the final version.

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ABSTRACT

Aims: The aim of this study was to assess the impact of hemoglobin polymorphisms and G6PD deficiency on the course of uncomplicated malaria infection in children aged from 2 to 10 years in Burkina Faso.

Study Design: The study was conducted as a longitudinal study in Banfora health district. A total of 150 children aged from 2 to 10 years was enrolled and followed up between May 2015 and February 2016. Blood samples were collected at four different time points: before infection (Visit 1), during asymptomatic parasitemia (Visit 2), during symptomatic parasitemia (Visit 3) and three weeks after treatment (Visit 4). Clinical examination, hematology parameters and malaria diagnosis using microscopy were performed. Hemoglobin and G6PD typing were done using PCR-RFLP. Hemoglobin AA genotypes were defined as normal hemoglobin while Hemoglobin AC, AS and SS were defined as abnormal hemoglobin (hb non-AA).

Results: The prevalence of hemoglobin (hb) genotypes was 81.21% for AA while hb non-AA genotypes were estimated at 18.79% (12.08% for hbAC, 6.04% for hbAS and 0.67% for HbSC). The prevalence of G6PD genotypes was 89.26% and 10.74% for normal G6PDn and G6PD deficiency respectively. The prevalence of asymptomatic carriers of *P. falciparum* was not affected neither by the genotypes of Hemoglobin, nor by the G6PD deficiency. Conversely, the risks of developing uncomplicated malaria in G6PD deficiency (G202A) group, was significantly lower (p = 0.04).

The results showed a significant difference (p<0.0001) in the means of *P. falciparum* parasite densities between asymptomatic and symptomatic phase in Hemoglobin AA genotypes carriers while the means of parasite density was comparable in non-Hemoglobin AA carriers.

Conclusion: Our study showed that G6PD deficiency protects against clinical malaria while *P. falciparum* parasite density increasing was correlated with carrying hemoglobin genotypes AA.

Keywords: Hemoglobin; G6PD; Polymorphism; RFLP-PCR.

1. INTRODUCTION

The X-linked Glucose-6-phosphate dehydrogenase (G6PD) deficiency and hemoglobinopathies are wide-spread human erythrocyte genetic diseases affecting millions of people [1,2]. The geographical distribution of hemoglobinopathies and G6PD deficiency is closely related to the present or the past malaria world distribution [3]. Individuals with these disorders showed reduced malaria infection rates, disease severity or parasitic burden [4,5]. Sickle-cell hemoglobin (HbS) and hemoglobin C (HbC) are both caused by point mutations in the beta globin gene of hemoglobin. Sickle-cell hemoglobin (HbS) is caused by a single amino acid substitution at position 6 of beta globin. The sickle cell mutation (henceforth ‘βS’) replaces glutamic acid with valine. Hemoglobin S (HbS) has become a stable polymorphism within malaria endemic regions, associated with limited life expectancy among homozygous individuals who suffer from sickle cell disease, and extended life expectancy of heterozygous individuals who are more likely to evade malaria [6,7]. Hemoglobin C (HbC) is caused by a mutation (henceforth ‘βC’) in the 6th position of the amino acid sequence of beta globin, where the glutamic acid is substituted by the lysine. HbAS is widely known to confer significant protection from severe and uncomplicated malaria [8,9] although underlying mechanisms are not precisely defined. Similar protection conferred by hemoglobin C (HbC) was recently suggested although the findings are less conclusive from clinical studies performed in Nigeria and Mali where no protection was found [10,11].

In Burkina Faso, sickle cell disease prevalence is estimated between 8 and 10% [12,13]. In a previous study its was found that AC or AS Hb genotypes was associated with lower risk of clinical malaria relative to normal genotype among children aged from one to three years [13].

The deficiency in G6PD is prevalent in Africa, in the Middle East, the Mediterranean and the Southeast Asia [4]. Among the approximately 200 G6PD variant alleles that have been described in the world, G6PD A- variant (G202A/A376G) predominates in sub-Saharan Africa region where the variant affects 15 to 20% of the African population [14]. In Burkina Faso,
the prevalence of G6PD is estimated between 8 and 9%. With respect to gender, there was a significantly higher prevalence of hemizygous male (15.5%) than homozygous female (4.4%) since the disease is X-linked. Among people with G6PD deficiency in this study, the G6PDA-(202A/376G) was the most common variant observed in 88.9% of deficiency cases. In a previous study in Burkina Faso, it is showed that the 202A/376G G6PD A- was the only deficient variant detected [15].

As such, G6PD deficiency has long been hypothesized to be a target of positive selection owing to partial protection afforded against malaria [16]. Nevertheless, epidemiological studies investigating this hypothesis have yielded conflicting results that do not support this conclusion.

The aim of this study was to investigate the relationship between hemoglobin genotypes, G6PD deficiency and the occurring of the uncomplicated malaria infection in children aged 2-10 years old living in Banfora health district area.

2. METHODOLOGY

2.1 Study Sites

The study was conducted in two villages (Nafona and Bounouna) located in the Banfora Health District area, in the province of Comoé, at 441 km west of Ouagadougou, the capital city of Burkina Faso. The entire health district of Banfora is organized in 24 health communities’ clinics. Malaria transmission is also markedly seasonal and is intense during the rainy season (May - November). The cumulative annual entomological inoculation rate varies from 55 to 400 infective bites/person/year [17]. P. falciparum is the main parasite present in more than 90% of infections. The incidence rate of uncomplicated malaria in less than five year old children is 1.18 episodes/child-year at risk and around 60% of the total annual number of malaria episodes occurs during the high malaria transmission season [17].

2.2 Patients, Study Design and Sampling

This study involved 150 children aged between 2 and 10 years, males and females who did not show any symptom of malaria infection at the time of recruitment. The design of the study consisted of a longitudinal follow up and sampling of children four times for a period of up to 10 months. Briefly, only children without P. falciparum infection (visit 1), with no chronic disease and no clinical sign of other infections were enrolled. Enrolled children were then weekly followed by study nurses with a check of body temperature and the presence of the malaria parasite in the blood by using malaria rapid diagnostic test (SD Bioline Malaria Ag P.f/Pan, SD STANDARD DIAGNOSTIC, INC) (MRDT). Asymptomatic carrier’s participants defined as having a positive MRDT and no fever (T°<37.5) were referred to the clinical research unit in Banfora where thick and thin blood smears were performed to confirm the presence of P. falciparum (Visit 2). Participants were then invited to visit the clinical research unit as soon as an episode of malaria (fever) occurs. The third sampling (Visit 3) was performed when an episode of malaria infection occurred. In addition to the MRDT, onto one slide a thick and thin blood smears were performed from participants with a positive MRDT and with a body temperature ≥37.5°C. Antimalarial treatment was provided free of charge to those having positive MRDT following the national guidelines for malaria treatment. Finally, the last sampling at Visit 4, was conducted three weeks after malaria treatment. Visit 4 is considered as the end of the follow up and thick and thin blood smears were performed to confirm the presence of P. falciparum.

Venous blood was also collected at each time point visit in EDTA tube for hematology, G6PD and hemoglobin typing.

2.3 Microscopic Malaria Diagnosis and Hematology Test

P. falciparum parasitemia was microscopically diagnosed. Blood slides with thin and thick blood smears were prepared and stained with Giemsa (10%). The thick film was fixed with methanol after drying. The parasites were counted against 200 leukocytes and then extrapolated to parasites per microliter of blood. At least one hundred power film fields were examined before assigning a negative malaria diagnosis. The number of parasites per microliter of blood was calculated using the last full blood count of the patient or the theoretical value of 8000 leucocytes/μl. The Parasite Density (PD) was estimated using the following formula:
PD = N x 8000/X
With N = number of parasites counted and X = number of counted leucocytes or the value of the full blood count.

Two experts microscopists who read each blood slide were blinded from each other’s reading. In the event of a discrepancy between the two readers, in terms of species, presence or absence of malaria parasites, or if parasite densities differed by more than 30%, the slide was re-examined by a third laboratory technician. Arithmetic mean of the two closest readings was used as the final value for parasite density. A slide was considered negative if no parasites were found after 100 HPF were examined.

*P. falciparum* asymptomatic carriers was defined as a detection of at least one trophozoite on the microscopy field while the clinical malaria was defined as the presence of *P.falciparum* trophozoites associated with an axillary temperature $\geq 37.5^\circ C$.

One milliliter of venous blood was analyzed to provide the full blood count for each participant by using an hematology automated instrument (ABX Pentra 60 or Cell Dyn 200) according to an internal Standard Operatory Procedure and the manufacturer’s instructions.

### 2.4 Human Hemoglobin and G6PD Genotyping

Human DNA was extracted using a QIAGEN kit (QIAamp DNA blood mini kit). Hemoglobin and G6PD genetic variants were typed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). For hemoglobin, DNA samples were amplified using a 5’-AGG AGC AGG GAG GGC AGG A-3’ forward primer and a 5’-TCC AAG GGT AGA CCA CCA GC-3’ reverse primer. The PCR conditions used to amplify the fragments were: 96°C/5 min, 30 cycles of 96°C/30 s, 60°C/1min, and 72°C/30 s, and final extension of 72°C/5 min for regions including mutations 202 G>A (109 bp product). The 109-base pair bp fragment obtained was subjected to digestion with MnlI for discrimination of mutant type (63 pb, 46 pb). Digestion was carried out for three hours at 37°C and the products were run on a 3% agarose gel [12]. Normal group is composed of subject without A202G mutation. Deficient group is composed of subject presenting A202G mutation characterized by the presence of both 63 pb and 46 pb fragments.

According to hemoglobin typing and the analysis purpose, study participants were divided into two groups. Normal hemoglobin group (AA) and abnormal hemoglobin group (AS, AC, SC, CC, SS).

### 2.5 Data Management and Analyses

The data were double entered in a database using an Access 2007 program and analyzed using the software Stata IC version 11.1. Pearson $\chi^2$ tests and Fisher's exact test were used for comparison of proportions. Student and ANOVA tests were used for comparison of means. $P$ value $= 0.05$ was used as the threshold significance for the different statistical tests.

We first determined the prevalence of hemoglobin type and G6PD type in our study area. Secondly we compared asymptomatic parasite carriers and *P. falciparum* density, hemoglobin level and clinical malaria prevalence according to hemoglobin and G6PD types. At the end we carried out multivariate analyses to consider all the various confounding factors such as hemoglobin and G6PD type, parasite density, age and gender on the hemoglobin level.

In order to avoid biased inferences, the calculation of the Hardy-Weinberg equilibrium was made from the genotypes frequencies of the hemoglobin variants and then reported in the results section with the following assumption:
\[ p = \text{Frequency of A allele, } q = \text{frequency of S allele and } r = \text{frequency of C allele} \]

\[ p_2 = \text{AA frequency, } q_2 = \text{AS frequency, } r_2 = \text{CC frequency, } \frac{1}{2}pq = \text{AS frequency, } \frac{1}{2}pr = \text{AC frequency} \]

\[ p = \frac{(2AA + AS + AC)}{2N}; q = \frac{(2SS + AS + SC)}{2N}; r = \frac{(2CC + AC + SC)}{2N} \]

3. RESULTS

3.1 Study Population Basic Characteristic

The characteristics of the study population at the baseline are summarized in Table 1. The sex ratio (F/M) and the means age were statistically comparable between the two study villages with \( p \)-value corresponding to 0.8 and 0.5 respectively.

Among the study patients, 136 (91.3%) had *P. falciparum* uncomplicated malaria infection while 28 (18.8%) patients were asymptomatic *P. falciparum* parasite carriers. From the uncomplicated malaria cases treated according to the national malaria guideline, 41 (30.1%) of them were *P. falciparum* positive three weeks after the initiation of the treatment (Table 2).

Genotyping for hemoglobin and G6PD were unsuccessful from one sample. A total of 149 is then included in the subsequent genotyping analysis.

3.2 Hemoglobin Phenotype and G6PD Variant Distribution in Study Population

A comparison between observed number and theoretical number according to Hardy-Weinberg presented in Table 2 showed our population was in Hardy-Weinberg equilibrium with \( p \)-value=1.

The analysis of the hemoglobin genotypes distribution as presented in Table 3 showed the hemoglobin genotype HbAA recorded the statistically highest frequency (81.2%). This was followed by hemoglobin variant HbAC (12.1%), hemoglobin variant HbAS (6%), and HbSC (0.7%).

The distribution of G6PD genotypes in the study population displayed 89.3% with normal G6PD genotype (G202A) and 10.7% with G6PD deficiency genotype (A202G). There was no statistically significant difference in the distribution of hb genotypes and G6PD deficiency according to the gender (\( p \)>0.5) (Table 1).

3.3 Mean Parasite Density According to Hemoglobin Genotype and G6PD Type (Table 3)

At asymptomatic parasitemia phase no significant difference was observed in the *P. falciparum* parasite means densities between normal hemoglobin 4743 (1981-7505) tf/µl and abnormal hemoglobin carriers 8770 (597-16942) tf/µl (\( p = 0.3 \)), between normal G6PD carriers 5920 (2601-9239) tf/µl and deficient G6PD carriers 1526 (197-2855) tf/µl (\( p = 0.2 \)). Though the parasite densities, in the overall study population, from asymptomatic phase [5606 (2502-8710)] tf/µl increased significantly during the symptomatic phase [55380 (42170-68590)] tf/µl and decreased three weeks after treatment [11489(4424-18553)] tf/µl in infected patients, there was no significant difference between, hemoglobin genotypes or G6PD deficiency on this variation of *P. falciparum* densities during the course of malaria infection. However, the results showed significant difference in the means of parasite densities between asymptomatic and symptomatic phase in Hemoglobin AA genotypes carriers (\( p = 0.0001 \)) while the means parasite density was comparable in Non Hemoglobin AA carriers (\( p = 0.08 \)). In both G6PD normal and deficiency group the parasite densities mean increased significantly (\( P = 0.00001 \)) from the asymptomatic to symptomatic phase.

Table 1. Basic characteristic of study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Village</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bounouna</td>
<td>Nafona</td>
<td>Total</td>
</tr>
<tr>
<td>Number of participants (%)</td>
<td>72 (48)</td>
<td>78 (52)</td>
<td>150 (100)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female: n (%)</td>
<td>33 (45.8)</td>
<td>37 (47.4%)</td>
<td>70 (46.7%)</td>
</tr>
<tr>
<td>Male: n (%)</td>
<td>39 (54.2)</td>
<td>41 (52.6%)</td>
<td>80 (53.3%)</td>
</tr>
<tr>
<td>Sex ratio (F/M)*</td>
<td>0.85</td>
<td>0.90</td>
<td>0.88</td>
</tr>
<tr>
<td>Means age (Years) **</td>
<td>3.9 (3.6-4.2)</td>
<td>4.3 (3.9-4.7)</td>
<td>4.2 (4.0-4.4)</td>
</tr>
</tbody>
</table>

*Sex ratio (Bounouna vs Nafona): \( p = 0.8 \): **Means age (Years) (Bounouna vs Nafona): \( p = 0.5 \):
Table 2. Test de hardy Weinberg

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed number</th>
<th>Observed frequency</th>
<th>Calculated frequency</th>
<th>Calculated number</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>121</td>
<td>0,812</td>
<td>0,815</td>
<td>121,43</td>
</tr>
<tr>
<td>AS</td>
<td>9</td>
<td>0,016</td>
<td>0,061</td>
<td>9,09</td>
</tr>
<tr>
<td>AC</td>
<td>18</td>
<td>0,121</td>
<td>0,115</td>
<td>17,4</td>
</tr>
<tr>
<td>SC</td>
<td>1</td>
<td>0,007</td>
<td>0,004</td>
<td>0,6</td>
</tr>
<tr>
<td>SS</td>
<td>0</td>
<td>0</td>
<td>0,001</td>
<td>0,15</td>
</tr>
<tr>
<td>CC</td>
<td>0</td>
<td>0</td>
<td>0,004</td>
<td>0,6</td>
</tr>
</tbody>
</table>

P=1

3.4 Mean Hemoglobin Level According to Hemoglobin and G6PD Types

Data presented in Table 3 showed that the means of hemoglobin level (95% CI) were comparable between normal and abnormal hemoglobin carriers (ANOVA, p > 0.05) at different phase of malaria course: asymptomatic phase, symptomatic and even after treatment.

Our data also showed that the deficiency has no influence on hemoglobin level (95% CI) with regard to the different steps of malaria course. There was no statistically significant difference when comparing the change in hemoglobin level between asymptomatic and clinical phase as a function of hemoglobin type and G6PD type (P > 0.05).

3.5 Clinical Malaria Prevalence According to Hemoglobin Genotype and G6PD Type

The prevalence of clinical malaria according to hemoglobin type was 90.9% for normal hemoglobin carriers and 92.9% for abnormal hemoglobin carriers. There was no statistically difference according to hemoglobin type on the prevalence of clinical malaria (P=0.8) (Table 3).

The prevalence of clinical malaria according to G6PD type was 93% normal G6PD carriers and 75% for deficient G6PD carriers. The prevalence of clinical malaria was statistically lower in deficient G6PD carriers group than normal G6PD carriers group (P=0.04) (Table 3).

3.6 Correlation between Hemoglobin Genotype, G6PD Type and Parasitemia and Hemoglobin Level

We performed multiple regression analyses (Fig. 1) to examine the relationship between sex, age, G6PD, visit and parasitemia and hemoglobin level in normal hemoglobin carriers and abnormal hemoglobin carriers. From the multivariate statistical model (Fig. 1-A) the predicted against actual Y plots for hemoglobin level showed that the models fit best in the two groups: p<0.0001 for abnormal hemoglobin carriers and p=0, 0042 for normal hemoglobin carriers. The multivariate analyses showed opposite trends of hemoglobin level with evolution phase (visit) of malaria (p=0, 0039) (Fig. 1B) in normal hemoglobin carrier but there was no such correlation between hemoglobin level and visit in abnormal hemoglobin carriers. The multivariate analysis also showed that the hemoglobin level is significantly higher in normal G6PD carriers than in G6PD deficiency carriers in abnormal hemoglobin carrier (p=0, 0003) in clinical phase of malaria (V3) (Fig.1C) and the hemoglobin level lower in children under five in normal hemoglobin group (p<0.001).

4. DISCUSSION

4.1 Prevalence of Abnormal Hemoglobin and G6PD Deficiency in the Study Area

The abnormal hemoglobin prevalence in our study was 18.79%. The prevalence of abnormal hemoglobin observed in this study area was lower than those found in previous studies carried out in others localities in Burkina Faso. Indeed, in Ouagadougou, the capital city located at the central part of the country Bougouma et al [13] found a prevalence of 26, 8% of abnormal hemoglobin in Saponé at the southern part of the capital city. The low prevalence of abnormal hemoglobin in our study compared to the others studies carried out in Burkina Faso could probably be explained by a geographical difference as this is the first known study carried at the southwest part of the Burkina Faso. Moreover, the study did not found any carriers of homozygotes CC and SS but found a low
Table 3. *P. falciparum* carriers and malaria parasites densities according to the hemoglobin type and G6PD deficiency

<table>
<thead>
<tr>
<th>Visit 1 (enrolment) n (%)</th>
<th>Total</th>
<th>HbAA</th>
<th>Non AA</th>
<th>p-value</th>
<th>G6PDn</th>
<th>G6PDd</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>80 (53.7)</td>
<td>64 (80.0)</td>
<td>16 (20.0)</td>
<td>70 (87.5)</td>
<td>10 (12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female n (%)</td>
<td>69 (46.3)</td>
<td>57 (82.6)</td>
<td>12 (17.4)</td>
<td>63(91.3)</td>
<td>6 (8.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>10.9 (9.5-12.3)</td>
<td>10.9 (10.7-11.1)</td>
<td>10.8 (10.5-11.2)</td>
<td>9.8 (10.7-11.1)</td>
<td>10.9 (10.4-11.40)</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

Visit 2 (Asymptomatic parasitemia)

<table>
<thead>
<tr>
<th>P. falciparum carriers (%)</th>
<th>28/149 (18.8)</th>
<th>22/121 (18.2)</th>
<th>6/28 (21.4)</th>
<th>0.4</th>
<th>26/133 (19.5)</th>
<th>2/16 (12.5)</th>
<th>0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum density (tf/μl)</td>
<td>5606</td>
<td>4743</td>
<td>8770</td>
<td>0.3</td>
<td>5920</td>
<td>1526</td>
<td>0.2</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>10.7 (10.4-11.0)</td>
<td>10.7 (10.2-11.1)</td>
<td>10.8 (10.5-11.2)</td>
<td>0.6</td>
<td>10.7 (10.4-11.0)</td>
<td>10.4 (10.0-10.8)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Visit 3 (Symptomatic parasitemia)

<table>
<thead>
<tr>
<th>P. falciparum carriers (%)</th>
<th>136/149 (91.3)</th>
<th>110/121 (90.9)</th>
<th>26/28 (92.9)</th>
<th>0.5</th>
<th>124/133 (93.2)</th>
<th>12/16 (75)</th>
<th>0.04</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum density (tf/μl)</td>
<td>55380</td>
<td>54432</td>
<td>56028</td>
<td>0.9</td>
<td>56497</td>
<td>36558</td>
<td>0.5</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>10.7 (10.4-11.0)</td>
<td>10.3 (10.0-10.5)</td>
<td>10.8 (10.2-11.3)</td>
<td>0.1</td>
<td>10.4 (10.2-10.7)</td>
<td>10.4 (9.9-10.9)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Visit 4 (Three weeks after treatment)

<table>
<thead>
<tr>
<th>P. falciparum carriers (%)</th>
<th>41/149 (27.5)</th>
<th>36/121 (29.8)</th>
<th>5/28 (17.9)</th>
<th>0.1</th>
<th>37/133 (27.8)</th>
<th>4/16 (25)</th>
<th>0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum density (tf/μl)</td>
<td>11489</td>
<td>12930</td>
<td>1112</td>
<td>0.04</td>
<td>11714</td>
<td>9410</td>
<td>0.8</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>10.4 (10.2-10.6)</td>
<td>10.7 (10.4-10.9)</td>
<td>10.8 (10.4-11.2)</td>
<td>0.3</td>
<td>10.6 (10.4-10.8)</td>
<td>10.4 (9.8-11.0)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*AC: 18/149 (12.1%) AS: 9/149 (6%) SC: 1/149 (0.7%); *AC: 4/18 (22.2%) AS: 2/9 (22.2%) SC: 0/1 (0%); ** AC: 16/18 (88.9%) AS: 9/9 (100%) SC: 1/1 (100%)* **All the five (5) non AA were AC carriers** *Significant increasing in parasite densities means from asymptomatic to symptomatic phase (p<0.0001)
prevalence of SC (only one participant). We noticed as well a prevalence of hbAC genotypes higher than hbAS genotypes in our study. Consistent findings were described previously.[13,18]. However, from study conducted in Ghana, Kreuels B et al found that hemoglobin hbAS carriers was high than hemoglobin AC carriers[19]. The higher prevalence of hbAC in our study population can be explained by the fact that Burkina Faso is known as the epicenter of hemoglobin C [11].The C hemoglobin epicenter is in Mossi central tray where there is the maximal prevalence in this region [19]. The great disparity in the distribution of the abnormal hemoglobin confirms a such geographical variation. As a consequence, and getting a global map of this genetic disorder, there is a need for large sampling and analysis at the country level to capture the global overview of hemoglobin type distribution. In addition, the above studies carried out in Burkina Faso, included children less than five years of age, while our study included children aged 2 to 10 years. Due to the high mortality of children with hemoglobinopathies, the prevalence of hemoglobinopathy declines after the age of five. This could also partially explain our results. Also, awareness of the population about sickle cell disease can explain, the reduction in the prevalence of hemoglobinopathies carriers by limiting consanguineous wedding [20].

The prevalence of G6PD deficiency (10.5%) in our study was comparable to the 9.5% reported in Ouagadougou [21], and 9.0% reported in six African countries [21], but lower than the prevalence of 16.3% from others studies carried out in Burkina Faso [22]. These variations could be explained by the small size of our study sample.

The influence of hemoglobinopathies and G6PD deficiency on the physiopathology of malaria has been mentioned in numerous studies[12,13,15]. The understanding of the prevalence of these genetic disorders is very important because they may influence epidemiological data and especially help in interpreting the conclusions of clinical trials of vaccines as well as antimalarial drugs assessment [23].

4.2 The Impact of G6PD Deficiency and Hemoglobin Genotypes on
P.falciparum Infection

The comparison of the mean parasite density and the hemoglobin level at the asymptomatic phase, showed that parasite density increased significantly in normal hemoglobin group, normal G6PD group and deficient G6PD group. Conversely, in abnormal hemoglobin group, this increasing was not statistically significant suggesting that abnormal hemoglobin did not offer any well condition to P. falciparum multiplication and then may contribute to his limitation. The mechanism by which abnormal hemoglobin inhibit parasite multiplication are not yet clear. However, they exist models currently proposed in the literature that include reduced disease-mediated cytoadherence of parasitized hemoglobinopathic erythrocytes, impaired intraerythrocytic development of the parasite, dampened inflammatory responses. Our result are similar with some previous reports [24].

We have also noted that the prevalence of clinical malaria was statistically lower in deficient G6PD carriers group than normal G6PD carriers group suggesting that G6PD deficiency protect against malaria clinical symptoms appearance. Impaired growth of parasites in G6PD-deficient erythrocyte in vitro has been reported in some studies, but not in others. In a previous report, Marina Cappadoro et al, 1998, it was showed that G6PD deficient erythrocyte parasitized were phagocytosed 2.3 times more intensely than normal [25]. In a previous study in Burkina Faso, Ouattara et al (2014) showed that G6PD deficiency protect against asymptomatic malaria [15]. The same result was found in Gabon [26,25]. In a systematic review, [27] it was mentioned that that G6PD deficiency can potentially protect against uncomplicated malaria in African countries, but not severe malaria [27].

4.3 Relative Effect of Multifactorial Factors Hemoglobin Genotype, G6PD Type, Parasitemia and Hemoglobin Level on the Course of Malaria Infection

A multivariate analysis showed that abnormal hemoglobin carriage may protect against hemoglobin level declining during malaria evolution. Anemia, a lower rate of hemoglobin, is one of the criteria of severe malaria so our result confirmed that abnormal hemoglobin protects against severe malaria. Indeed a similar confirmation was done by Agarwale et al showing that HbC did not protect against infection or uncomplicated malaria but could protect against severe malaria in the Dogon population of Bandiagara [28]. In 2015, in Nigeria
Fig. 1: Association of hemoglobin level with visit, age, sex and G6PD in Normal and Abnormal hemoglobin groups. (A) Full multivariate models (Normal hb group, left panel, and Abnormal hb group, right panel). (B,C,D,E) Univariate models testing the association of each parameter (Normal hb group, left panels, Abnormal hb group, right panel) according to visit number (B), G6PD (C) Sex (D) and Age (E). The red solid line shows the line of fit, the red dashed line represents the 95% confidence curves and the blue dashed line shows the horizontal mean reference that represents the null hypothesis.
Igbenehgu et al. reported a strong protection of HbAS and HbAC genotypes against asymptomatic *P. falciparum* [29]. Protection of HbAC carriers against clinical forms of *P. falciparum* malaria has also been reported by Mangano et al. [30] in Burkina Faso that HbAS genotype was associated with a 70% reduction of parasite *P. falciparum* unlike HbAC carriers, although a strong protection was also observed in HbCC and HbSC subjects [28]. Our results suggest that even if haemoglobinopathies S or C protect against severe forms of malaria like G6PD deficiency, they do not confer protection against *P. falciparum* infections [31] with regard to the prevalence of malaria infection in our study population.

5. CONCLUSION

In this study we found that carrying the abnormal hemoglobin reduced the *P. falciparum* parasite multiplication from asymptomatic to symptomatic phase. The study also concluded that abnormal hemoglobin prevents the reduction of the hemoglobin level. Clinical malaria prevalence was statistically lower in G6PD deficiency group than G6PD normal. Clinical trial on malaria in endemic regions should consider Hb genotypes and G6PD deficiency as a potentially important confounder, particularly among young children. The disparity of the prevalence of the abnormal hemoglobin observed from the different studies carried out in Burkina Faso, suggest a large national mucking regarding the genotyping of the hemoglobin as well as the G6PD deficiency in order to provide the country with a full set of data on these common human disorders.

CONSENT AND ETHICAL APPROVAL

This study was approved by the Ethical Committee of the Ministry of Health of Burkina Faso (Ministry of Health, Burkina Faso; protocol number 2015-02-018) and the Institutional Review Board of New York University Abu Dhabi (UAE, protocol number 011-2015). Written informed consent was obtained from the parents or legal guardians of all participating children prior to enrolment. In case of any health concerns, the study participant was treated free of charge according to the local standards of care.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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