Sickle Cell Anemia and Glucose-6-phosphate dehydrogenase (G6PD) deficiency: Impact on Biological and Clinical Parameters

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Abstract — Both sickle-cell anemia and G6PD deficiency are red blood cell gene abnormalities that cause hemolytic anemia. Their association may have serious consequences. The objective of this study is to determine the prevalence of such an association at the CNRD of Niamey and the impact of G6PD on sickle-cell anemia patients. A prospective cross-sectional study was conducted with 227 major sickle-cell anemia patients in stationary phase by comparing hematological parameters, free and total bilirubin concentrations, impaired hepatic and splenic functions as well as hospitalization and blood transfusion rates. G6PD intra erythrocytic was determined by spectrophotometry at all sickle cell patients on the hemolysate of washed red blood cells. Findings were analyzed by Epi-info 3.5.4 and the significance level was set at P < 0.05.

G6PD was found in 7.08% of sickle-cell anemia patients. SS sickle-cell anemia was the most frequent form of the disease (83.3%). Hb levels as well as reticulocyte rates and MCHC were not significantly different in both groups of major sickle-cell anemia patients. But, the level of total bilirubin was significantly high in sickle-cell anemia in case of G6PD deficiency. Though the risk of hospitalization was similar in both groups of patients, sickle-cell anemia patients with G6PD deficiency were more likely to develop hepatomegaly or splenomegaly.

Keywords — Sickle cell anemia, G6PD deficiency, Hemoglobin, clinical parameters, Niamey

I. INTRODUCTION

Sickle-cell anemia and Glucose-6-phosphate dehydrogenase (G6PD) deficiency are two red blood cell gene abnormalities found in Sub Saharan Africa, especially in Niger with high rates of incidence. Each of these abnormalities causes acute and chronic hemolytic anemia. G6PD is the key enzyme in the pentose pathway which protects red blood cells against oxidative stress and produces part of the energy needed by red blood cells. Subjects with major sickle cell anemia syndrome experience multiple severe infections, acute vaso-occlusive and hemolytic crises. The relative higher incidence of both pathologies in our sub-continent [1] is attributable to their relative protection against malaria [2]. Use of some drugs may generate serious complications in some patients. [3]

Some authors believe that G6PD deficiency has little or no impact on sickle-cell anemia [4], whereas others are of the view that this deficiency worsens biological and clinical prognosis [5]. Through this study, our goal was to assess the incidence of G6PD deficiency in the population of sickle-cell anemia patients treated on a regular basis at the National Sickle Cell Anemia Reference Center (CNRD) in Niamey and evaluate the impact of such an association on biological and clinical parameters and the level of blood transfusion use.

II. PATIENTS AND METHODS

Type of study

This was a 5 month (February to June 2015) long descriptive cross-sectional study.

Study Population

The study population included sickle cell anemia patients regularly registered and treated at the National Sickle Cell Anemia Reference Center of Niamey (CNRD).
Inclusion Criteria

Individuals with major sickle cell anemia syndrome (SS, SC, S/B thalassemia and S/ HPFH) in stationary phase and whose most recent blood transfusion goes back to at least 4 months were included in this study. The stationary phase is definition characterized by the absence of any fever, vaso-occlusive or hemolytic crises. Patients with sickle cell trait and those admitted on an emergency basis were excluded from this study. Adult patients who were involved in this study gave their informed consent and for minors to be involved, written parents’ consent was required.

Study Sample

Individuals were randomly selected during programmed consultations and the size of the sample was determined using the following formula: \[ N = \frac{\delta^2 x P (1-p)}{I^2} \].

Blood sampling

Whole blood was collected from each individual at elbow crease into an EDTA tube for the purpose of carrying out complete blood count (CBC), determining intra erythrocytic G6PD and into a dry tube for the purpose of determining total and direct bilirubin.

Complete blood count (CBC)

CBC was conducted using «Cell dyn 1800» type automated cell counter of Abbott diagnostics.

Determination of G6PD

Packed red blood cells were washed 3 times with physiological water before hemolysis and intra erythrocytic G6PD activity was determined spectrophotometrically at 350 nm using BIOREX Diagnostics Limited (UK) reagents on the hemolysates obtained. Tests were conducted in conformity with the instructions provided by the manufacturer of reagents.

Reticulocyte count

Reticulocytes were manually counted by microscope in thin smears obtained following blood incubation with brilliant cresyl blue, 1%, for 30 minutes.

Data Analysis

STUDENT t-test was used to compare mean quantitative variables and Chi-square test was used to compare qualitative variables. Significance threshold: P < 5%.

Ethical considerations

The study was approved by the National Ethics Committee and was clear of any conflicts of interest. Patients involved in this study were provided with enlightened information. Results are covered by the rule of medical confidentiality.

III. RESULTS

The National Sickle Cell Anemia Reference Center (CNRD) records 3,576 sickle cell anemia patients followed up on a regular basis, including 227 patients included in ours sample, i.e., a participation rate of 6.34%. The average age of patients in our sample was 10.39 years ± 0.59. Gender breakdown shows 52.40% of female patients and 47.60% male patients. It is estimated that the parents of 32.75% of sickle cell anemia patients in our sample were blood related. The major sickle cell anemia syndrome most frequently found was the SS type (83.3%), followed by the SC type with 13.7%.

Table I: Sickle cell anemia profile

<table>
<thead>
<tr>
<th>Sickle cell status</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>189</td>
<td>83.3%</td>
</tr>
<tr>
<td>SC</td>
<td>31</td>
<td>13.7%</td>
</tr>
<tr>
<td>S/β°thalassemia</td>
<td>5</td>
<td>2.2%</td>
</tr>
<tr>
<td>S/PHHF</td>
<td>2</td>
<td>0.9%</td>
</tr>
<tr>
<td>Total</td>
<td>227</td>
<td>100%</td>
</tr>
</tbody>
</table>

G6PD deficiency was found in 16 sickle cell anemia patients in our sample (7.08%), including 0.88% of total deficiency and 6.20% of partial deficiency. G6PD activity was on average 4.41 ± 0.44 IU/g Hb in deficient sickle cell anemia patients against 18.90 ± 0.46 UI/g Hb in non G6PD deficient sickle cell anemia patients.

Table II compares hematological and hemolytic data on sickle cell anemia patients with or without any G6PD deficiency. Hb concentration, as well as reticulocyte rates and CCMH were not significantly different in both groups of patients. The level of total bilirubin was significantly higher in deficient sickle cell patients than in non deficient patients.
Table II: Hematological and hemolytic characteristics according to G6PD activity

<table>
<thead>
<tr>
<th>Biological parameters</th>
<th>G6PD activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>7.72 ± 0.10</td>
</tr>
<tr>
<td>CCMH</td>
<td>32.30 ± 0.08</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
<td>14.95 ± 0.69</td>
</tr>
<tr>
<td>BT (mg/dl)</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>BD (mg/dl)</td>
<td>0.49 ± 0.03</td>
</tr>
</tbody>
</table>

*P< 0.05

Table III reports the rates of hospitalization and blood transfusion in both groups of patients. This involves hospitalizations recorded at least once during the last 12 months prior to the study. Hospitalization rate was 87.5% in the group of G6PD deficient against 86% in the group of non-G6PD deficient patients for the same period. Though quite low, the Relative Risk (RR = 1.01) of G6PD deficient patient’s hospitalization does exist as it stands at 1.01. The risk of hospitalization was 1.01 time higher in GPD deficient patients than in non-GPD deficient individuals.

31.2% of G6PD deficient patients received blood transfusion at least once in the last 12 months prior to the study against 51.6% of sickle cell patients with normal G6PD activity. In relation to such blood transfusion, the relative risk (RR) was 0.60. This suggests a protective effect against iterative transfusions. In fact, the very rapid disappearance of red blood cells attributable to the very fact that enzymatic activity was absent would translate into accelerated production of young cells when nutritional conditions are met.

Table III also revealed that the risk of hepatomegaly or splenomegaly was more significant in GPD deficient patients than in non deficient patients. In fact 18.8% of sickle cell deficient G6PD had a hepatomegaly against 12.7% in non-deficient. Similarly 37.5% of sickle cell deficient G6PD had a splenomegaly against 29.4% in non-deficient. The relative risk of splenomegaly was 1.3 and the risk of hepatomegaly was 1.5. G6PD deficient sickle cell patients were more likely to develop such organomegalies.

Table III: Impact of G6PD deficiency on the clinical parameters of sickle cell patients

<table>
<thead>
<tr>
<th></th>
<th>G6PD Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficiency</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>Yes: 14 (87.5%)</td>
</tr>
<tr>
<td></td>
<td>No: 2 (12.5%)</td>
</tr>
<tr>
<td>Transfusion</td>
<td>Yes: 5 (31.2%)</td>
</tr>
<tr>
<td></td>
<td>No: 11 (68.8%)</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>Yes: 3 (18.8%)</td>
</tr>
<tr>
<td></td>
<td>No: 13 (81.2%)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>Yes: 6 (37.5%)</td>
</tr>
<tr>
<td></td>
<td>No: 10 (62.5%)</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

In Niger, the incidence of sickle cell trait type is estimated at 25% in schools [6]. The average age of patients involved in our study was 10.39 years with extremes of [1 - 25 years]. This is a rather young age that could be explained by the age pyramid of Niger’s population and the recent initiation of CNRD activities. In fact, the population under 15 years accounted for 48 % of the general population in 2001 [7]. Gender breakdown of sickle cell anemia was not significantly different. Homozygous form SS was the major sickle cell syndrome found. This is the most common form, though hemoglobinosis C coexists in the Western part of the country.

The prevalence of G6PD deficiency among major SCD was 7.02% up from 11.8% in another study on umbilical cord blood of newborns alive [8]. One explanation for this large difference is the high rate of perinatal and infant mortality. Indeed, 51 on thousand live births do not celebrate their first anniversary according to data from the Demographic Survey of 2012 and that the overall risk of death between birth and the fifth birthday is estimated at 127 per thousand live births is nearly a eight children [7]. The prevalence that we found in this study is lower than that stated (21.6%) in Senegal Diop et al. [9] and in Togo GBADOE et al [10] with 24.1%.

G6PD activity tends to be more important in sickle cell patients because of the accelerated renewal of red blood cells related to the chronic hemolysis.
sustained by such patients. This activity was 4.41 UI/g Hb in sickle cell patients and 18 UI/g Hb in non deficient sickle cell patients. A study conducted at the National Blood Transfusion Center of Niamey showed that the level in G6PD deficient blood donors was 2.9 UI/g Hb against 11.7 UI/g Hb (data not shown) in non deficient ones. According to a study recently conducted by Benkerrou et al [5] on a cohort of very young children with major sickle cell anemia, the average GPD activity was 1.5 and 15 UI/g Hb respectively in GPD deficient patients and non deficient ones.

Analysis of hematological parameters showed that Hb level was slightly lower in G6PD deficient patients, though the gap was not significant. Results similar to our own [11], [12] showed that hemoglobin level in deficient patients was lower than the level in non deficient patients. MCHC was almost identical in both groups of patients we studied. G6PD deficiency did not change the number of GR, MCV, MCH as well as MCHC in sickle cell patients [13] et [5]. Reticulocyte rates rank among the biological parameters used to measure the level of medullary activity. In our sample, such rates were almost identical in both groups of patients with or without any G6PD deficiency, without any significant differences, as also shown by Simpore et al [14] following another study, suggesting the lack of strong influence exercised by this enzymopathy on sickle cell disease in stationary phase. Benkerrou et al [5] found on the one hand that the reticulocyte rates were significantly higher in G6PD deficient sickle cell patients, and on the other hand, lower Hb concentrations.

The study on the degree of hemolysis showed that total bilirubin concentration was higher in deficient sickle cell patients than in non deficient ones, suggesting however, slight aggravation of their chronic hemolysis in stationary phase. Such results are similar to those found by Benkerrou et al [5]. Controversies noted regarding the findings of studies on GPD and sickle cell anemia association may be linked to a number of factors, including the type of longitudinal or descriptive study, the age of patients et progressive stage of the disease.

Findings made on some clinical events by various authors were also divergent. According to Diop et al. [9], there is no difference in the occurrence of infections, vaso-occlusive crises or in transfusion between the two groups of patients. This finding was shared by Benkerrou et al [5] regarding only infectious episodes and vaso-occlusive crises. However, they found that anemia episodes were 3 times higher in G6PD deficient children and transfusions were also more important and repetitive than in non deficient children. Such findings on transfusion conflicted with our own findings, as only 31.2% of GPD deficient patients were transfused against 51.6% of non deficient patients with a relative risk (RR) of 0.60. In addition, in our sample, the number of hospitalizations in both groups was quite similar with a relative risk of 1.01, which indicated that such an association does not significantly increase the number of hospitalizations of GPD deficient sickle cell patients.

Findings noted in our study on splenomegaly and hepatomegaly conflicted with the findings of Diop et al [9] and Simporé et al [14]. The incidences of splenomegaly and hepatomegaly were respectively 37.5% and 18.8%. The relative risk for splenomegaly was 1.3 and for hepatomegaly, 1.5. For both clinical signs, the relative risk was quite higher in GPD deficient individuals than non deficient sickle cell patients. Our results were in total contradiction with the findings of Steinberg et al [4] who studied Black American patients and who showed that G6PD deficiency had no impact on the clinical signs of major sickle cell anemia. In our view, such a statement should be qualified taking into account the socioeconomic conditions of patients. Indeed, in developing countries facing pandemic infections, including malaria, parasite and bacterial infections and use of drugs, the emergence of such organomegalies is attributable to exaggerated solicitation of these organs.

V. CONCLUSION
This study showed that the prevalence of GPD deficiency was 7.08% in sickle cell patients at the CNRD. G6PD deficiency has no significant impact on hematological parameters of sickle cell anemia at stationary phase and on the level of hospitalization. However, the risk of organomegaly is higher in case of sickle cell anemia and GPD deficiency association. G6PD deficient sickle cell patients find themselves in a precarious balance situation which requires particular monitoring.

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CONFLICT OF INTEREST:
The authors declare having no conflict of interest regarding this article.
REFERENCES


