Glucose 6-Phosphate Dehydrogenase Deficiency and Sickle Cell Anemia: Frequency and Features of the Association in an African Community

By Ulrich Bienzle, Olugbemiro Sodeinde, C. E. Effiong, and L. Luzzatto

The glucose 6-phosphate dehydrogenase (G6PD) genotype was determined in 100 male patients with homozygous sickle cell anemia (SS) by a combination of quantitative assay, cytochemical testing, and starch-gel electrophoresis. Of the 100 patients tested, 16 were found to be G6PD deficient (GdA-), and 84 G6PD normal (22 GsA and 62 GdA). This distribution of G6PD genotypes did not differ significantly from that observed in the general population. The level of G6PD activity in GdA- SS patients was nearly always higher than in G6PD-deficient subjects who did not have an associated hemolytic state, but it was nearly always lower than in G6PD-normal subjects. The clinical course of sickle cell disease, including the degree of anemia, was not milder in GdA- than in G6PD-normal patients but could not be proved to be significantly more severe. It was concluded that in this community the incidence of G6PD deficiency in sickle cell anemia was not greater than would be expected by chance, and there was no evidence that the coexistence of the GdA- gene in SS patients ameliorated their disease.

The possible relationship between glucose 6-phosphate dehydrogenase (G6PD) and hemoglobin S among various populations and in individual cases has been the subject of a number of reports. Lewis et al. from Ghana first suggested that the incidence of deficiency for this enzyme was higher in patients with Hb S than in the general population. More recently, Piomelli et al. have gathered evidence in favor of an association between homozygous sickle cell anemia and G6PD deficiency in an American community of African origin. In Nigeria and in Tanzania, it was previously shown in large series that the alleles of the Hb locus and of the Gd locus assorted independently, as expected. Data on patients with sickle cell disease were not included in our previous study in order to avoid the risk of Gd genotype misclassification in subjects with hemolysis. Indeed, it is well recognized that G6PD typing of these patients is made difficult by the fact that they usually have a relatively young erythrocyte population and therefore high levels of G6PD.

From a study of 100 male patients whose G6PD genotype was determined by a combination of four different methods, it is now established that there is no association between G6PD deficiency and sickle cell anemia in Nigeria. Fur-
Moreover, it has been found that the clinical course of the latter disease in this country is not ameliorated by coexistence of the former trait.

MATERIALS AND METHODS

The study was conducted on 100 male patients with homozygous sickle cell anemia (SS), in the age group from 10 to 25 yr, attending the outpatient clinic of the University College Hospital, Ibadan, Nigeria. The hemoglobin type was determined by paper and by starch-gel electrophoresis. Case of S-thalassemia were eliminated by determining the level of hemoglobin A2, which was always less than 3% in the subjects admitted to the study. The level of hemoglobin F was determined by the alkali denaturation technique, and it ranged from 4% to 20%.

The G6PD status was assessed by the following methods: (1) Fluorescence spot test. (2) Spectrophotometric quantitative assay as recommended by W.H.O. The normal range in our laboratory for subjects with G6PD type A and B lies between 6 and 10 IU/per g Hb, and for subjects with G6PD type A' it is below 1 IU/per g Hb. (3) Methemoglobin reduction cytochemical test according to Gall et al. The normal value for samples with G6PD (+) phenotype is below 6% eluted cells, and for samples with G6PD (−) phenotype it is above 80% eluted cells. (4) Starch-gel electrophoresis. From the results of this set of tests, each subject could be unambiguously assigned to one of the three genotypes GdA, GdB, or GdA'. In 28 cases, the Gd genotype was reassessed after an interval of 1 yr, and agreement with the original classification was found in all cases but one.

A semiquantitative assessment of the severity of sickle cell anemia was carried out in 30 patients (ten for each G6PD genotype), matched for age, who had attended the Hematology Clinic for at least 2 yr. The degree of anemia was expressed by determining the steady-state PCV, defined as the average of at least 6 packed cell volume (PCV) values, measured over a period of at least 1 yr, at intervals of at least 1 mo, excluding values obtained at times when there was evidence of infection, recent transfusion, or any crisis situation. In calculating the steady-state PCV, it was found that the mean and median PCV for each patient were the same within one hematocrit point, indicating that steady-state PCV was a very stable parameter. The steady-state PCV and other clinical parameters (see Table 2) were additively compounded to yield a score, on the basis of which patients were arbitrarily classified as having mild disease (score below six), moderate disease (score between six and ten), or severe disease (score above ten).

RESULTS

The classification of SS patients as G6PD normal and G6PD deficient, respectively, could be done unambiguously (Fig. 1) by the methemoglobin elution test, since all subjects were clearly resolved into two clusters, having fractions of eluted cells either below 6% [G6PD (+) phenotype] or above 48% [G6PD (−) phenotype]. There was almost no overlap in the quantitative G6PD values between the two groups (Fig. 1). The fluorescence spot test, not surprisingly, yielded false-normal results in the majority of SS patients with G6PD deficiency, presumably because of their young red cell population. From the electrophoretic data, the subjects with G6PD (−) phenotype were confirmed to be GdA-, and the subjects with G6PD (+) phenotype were subdivided into GdA.
G 6-PD DEFICIENCY AND SICKLE CELL ANEMIA

Fig. 1. Glucose 6-phosphate dehydrogenase genotypes in patients with sickle cell anemia. Each dot represents one patient. 0, GdA; a, Gdb; e, GdA. The shaded area at the bottom represents the range of G6PD activity found in G6PD-deficient subjects without acute or chronic hemolysis. The shaded area above represents the range of G6PD activity (mean ± 2 SD) found in G6PD-normal subjects without acute or chronic hemolysis. The G6PD activity (on the ordinate) is higher than the normal range in 63 out of 84 G6PD-normal SS patients, and it reaches the normal range in two out of 16 G6PD-deficient SS patients. Eluted cells (on the abscissa) clearly demarcate, without overlap, G6PD-deficient from G6PD-normal patients.

and GdA. We thus found that among the 100 SS patients, 16 were genetically G6PD deficient (GdA−), a slightly lower percentage than in the general population. The overall distribution of all three G6PD genotypes in SS patients did not differ significantly from that in the general population (Table 1).

The notoriously variable expression of sickle cell anemia, assessed as objectively as possible on the basis of at least 2 yr of follow-up, did not appear to be appreciably influenced by the G6PD genotype (Table 2); certainly it was not milder in the GdA− patients. This observation was also reflected in the values of the steady-state PCV which tended to be somewhat lower in G6PD-deficient patients, although the difference did not reach a level of statistical significance (Fig. 2). Similarly, the steady-state reticulocytosis (defined in a way analogous...
Table 2. Clinical Severity of Sickle Cell Anemia in Relation to G6PD Genotype

<table>
<thead>
<tr>
<th>Clinical Assessment</th>
<th>Number of Patients with G6PD Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GdA</td>
</tr>
<tr>
<td>Mild</td>
<td>5</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
</tr>
<tr>
<td>Average score</td>
<td>6.6</td>
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</tbody>
</table>

*The score for each patient was calculated by adding up the following points: (1) Steady-state PCV (see Materials and Methods); 20% or less, 6; 20%-25%, 4; 26%-30%, 2; over 30%, 1. (2) Liver enlargement below costal margin: more than 6 cm, 2; 2-6 cm, 1; less than 2 cm, 0. (3) Blood transfusion, 1 point per transfusion. (4) Osteomyelitis, 1 point per site involved. (5) Hospital admission, 1 point per each admission. (6) Subjective feeling, frequent complaints, 2; infrequent complaints, 1; mostly well, 0.

to steady-state PCV, see Materials and Methods) averaged 15% in five G6PD-deficient patients, but only 11% in nine G6PD-normal patients.

DISCUSSION

No association between sickle cell anemia and G6PD deficiency is to be expected a priori, since the two relevant genes are known to be located on two different chromosomes (Gd is sex linked, whereas Hb α is not), and they are found to assort independently in a population where both are very prevalent. Therefore, a difference in the frequency of G6PD deficiency among sickle cell anemia patients can only occur by postzygotic selection, indeed by postnatal selection, since the phenotypic expression of the homozygous S condition is not clinically significant until at least a few months after birth.

![Fig. 2. Effect of G6PD genotype on severity of anemia in SS patients. The difference in the distribution of steady-state PCV (see Materials and Methods) is not significant, but the values tend to be lower in GdA* patients.](image)
Some of the previous conflicting data on this issue (Table 3) can probably be explained on technical grounds. For instance, in a series from Ghana, G6PD activity was measured only by a semiquantitative method, and the finding of a high frequency of G6PD deficiency in sickle cell anemia was not confirmed in a subsequent report by the same author. Unfortunately, in the latter series, G6PD was only tested by electrophoresis without any attempt to quantitate the enzyme. In other reports, no association was found, but the reliability of G6PD genotypic classification by the techniques employed has been questioned, in view of the fact that G6PD activity of G6PD (−) subjects with a severe hemolytic disorder such as sickle cell anemia may fall within the normal range. In our own homozygous S G6PD-deficient patients, we confirmed the finding of Piomelli et al. that G6PD activity was regularly increased, although the values obtained were still below the normal range in about 80% of the cases. Thus, we conclude that there is indeed no association between G6PD deficiency and sickle cell anemia.

We consider it unlikely that the discrepancy between the data of Piomelli et al. and ours was due to technical causes, since the methodology employed was similar and the genotypic classification highly reliable in both series. The distribution of reticulocyte counts was also similar, although Piomelli et al. found the G6PD activity of G6PD-deficient patients falling within the normal range in a higher proportion of cases (38%). A difficulty in their study might have arisen from considerable genetic heterogeneity of the population tested. However, the possibility that the difference in incidence of G6PD deficiency was due to a sampling bias was made unlikely by showing that SS patients and AA subjects had the same frequency of the GdA gene, which is also of African origin, and which has a frequency similar to GdA in most African populations from which American blacks are thought to descend.

Since the present survey was completed, two papers on this topic have appeared in the United States. Beutler et al. have found, in the Los Angeles area, that the GdA genotype is not more common among SS patients than in their non-SS sibs, but it is more common, as in Piomelli’s series, than in a control population. Steinberg and Dreiling have found, in Mississippi, that the frequency of GdA− is no higher in adult SS patients with mild sickle cell anemia.
than in a control group. The slightly different results obtained in various parts of the United States may reflect heterogeneity in population structure and in patient selection. As far as a comparison between the U.S. and Nigeria is concerned, it is possible that $Gd^A$ gene may entail a relative increase in fitness for SS patients in New York, as postulated by Piomelli, but not in Ibadan, due to differences in the environment.

From our own clinical assessment (Table 2 and Fig. 2), the coexistence of the $Gd^A$ gene possibly causes a slight disadvantage to patients with sickle cell anemia in this community, and certainly it does not ameliorate the course of the disease. Examples of life-threatening intravascular hemolysis in G6PD-deficient SS patients have been described. For practical purposes, it is important to emphasize that, within the genetic and ecologic context of tropical Africa and within a relatively homogeneous population group, there is no evidence that G6PD deficiency favors survival of subjects with sickle cell anemia.

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