Effects of Glucose-6-Phosphate Dehydrogenase Deficiency Upon Sickle Cell Anemia

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We studied the interactions of the A" variety of glucose-6-phosphate dehydrogenase (G6PD) deficiency and sickle cell anemia (HbSS) to see if G6PD deficiency influenced laboratory and clinical features of HbSS. A total of 801 male patients over age 2 had G6PD electrophoresis on cellulose acetate membranes. Assays of both G6PD activity and hexokinase activity were then done on all samples that had an electrophoretic pattern other than the normal wild type (GdB). The collection of clinical data used a standardized protocol. Using cluster analyses we classified 10.4% males to be G6PD deficient, while 18.4% had the functionally normal GdA" enzyme. The prevalence of G6PD deficiency did not change significantly when age was stratified by decade, suggesting little survival advantage or disadvantage of the combination of G6PD deficiency and HbSS. Compared to patients who were not G6PD deficient, there were no significant differences in the hemoglobin concentration, mean corpuscular volume, reticulocyte count, bilirubin, or SGOT level in patients with HbSS who had G6PD deficiency. The incidence of painful episodes, sepsis, or acute anemic episodes was similar in both groups. Our results are consistent with recent studies of smaller numbers of patients that have found little influence of G6PD deficiency upon the course of HbSS. Specifically, we found no evidence that G6PD enhanced the severity of hemolysis or increased the incidence of acute anemic episodes or sepsis in HbSS.

BOTH SICKLE cell anemia (HbSS) and erythrocyte glucose-6-phosphate dehydrogenase deficiency (G6PD) are commonly found in black Americans. The interaction of these disorders has been of interest; different studies have suggested that G6PD deficiency has beneficial, deleterious, or no influence upon the course of HbSS. The Cooperative Study of Sickle Cell Disease (CSSCD) is studying the natural history of the severe sickle hemoglobinopathies. We have examined the effects of G6PD deficiency upon the clinical and hematologic features of a large number of patients with HbSS. We found little influence of G6PD deficiency upon the course of males with HbSS.

METHODS

Determination of Gd genotype. Venous blood was anticoagulated with acid-citrate-dextrose (ACD), mailed on wet ice to a central laboratory, and analyzed within ten days of collection. Erythrocytes were washed three times, with the buffy coat removed each washing, and G6PD electrophoresis was done on cellulose acetate membranes using the Helena Laboratories test kit. Normal (GdB) and deficient (GdA") samples were run with each set of patient samples. Assay of erythrocyte G6PD and hexokinase activity was done at 37°C using the method of Beutler, and assays were performed on all samples that upon electrophoresis had an enzyme with an electrophoretic mobility other than that typical of the GdB isozyme. With each group of G6PD and HK assays, samples from patients without sickle cell disease but with the GdA" variant, sickle cell disease patients with only the GdB enzyme, and normal controls (HbAA and GdB) were included.

In a subset of untransfused patients from a single clinic, blood was filtered through columns of microcrystalline cellulose and α-cellulose according to the technique of Beutler et al to remove leukocytes and platelets. In addition, the methemoglobin elution procedure was employed to cytochemically verify the presence of G6PD deficiency in this subset of patients.

Blood cell counts and erythrocyte indices were measured by Coulter electronic cell counters (Hialeah, FL). Reticulocytes were counted by standard techniques using a Miller ocular. Blood chemistries were measured by autoanalyzer methods. The blood counts and chemistries used for this analysis were obtained during a "steady state" intake clinic visit. Standard laboratory quality control practices were employed, and the comparability of tests among laboratories was continually monitored.

Painful episodes were said to occur when there was back, chest, extremity, or head pain that lasted at least two hours, was typical of previous painful episodes, and required a visit to the clinic, emergency department, or hospital. Pain associated with acute infections or other discrete acute events was excluded from this category. Sepsis was defined as a positive blood culture with or without evidence of a focus of infection.

An acute anemic episode occurred when a patient had a 30% reduction from the steady-state hemoglobin concentration or hematocrit that was not caused by blood loss or a transfusion reaction.

Statistics methods. A modified K-means cluster analysis modified according to an algorithm, was used to classify patients into a G6PD genotype of either deficient or nondeficient using hexokinase and G6PD activities as the classification variables. The purpose of this type of cluster analysis is to divide the population into K groups (for this setting K = 2) by minimizing the Euclidian distance from the mean of the groups to their members.

Several different methods for determining clusters were explored and evaluated. These included using both observed and standardized (mean = 0, SD = 1) values for G6PD activity and hexokinase activity, both including and not including the ratio of the two activities and choosing random and prespecified sets of initial points for two clusters (G6PD activity of 3 IU/gHb and hexokinase activity of 1.5 IU/gHb versus G6PD activity of 16 IU/gHb α hexokinase activity of 1.2 IU/gHb). Contour plots of GdA" and GdA" males were constructed based on means, variances, and covariances of
G6PD activity and hexokinase activity under the assumption that the distribution of observations is normal. Other statistical techniques used included: (1) a generalization to R by C contingency tables of the Mantel-Haenszel procedure for combining information from several 2-by-2 tables to evaluate overall association; (2) multivariate analysis of variance, a generalization of the analysis of variance to several dependent variables, which allows the correlations among the dependent variables to be used in the evaluation of the effects under consideration; (3) survival analysis using the log-rank and Gehan-Wilcoxon statistics to compare two survival distributions. 

In addition to the statistical techniques described above, we felt it was necessary to use a standardized value for the reticulocyte count. Differences among personnel at different clinics observed during quality control procedures led to this decision. In the analysis reticulocyte counts were expressed as standard scores:

\[ Z_{ik} = \frac{(X_{ik} - \bar{X}_j)}{S_j} \]

where \( Z_{ik} \) = the standard score for patient \( k \) in the \( i \)th clinic and the \( j \)th age group;

\( X_{ik} \) = the reticulocyte count for this patient;
\( \bar{X}_j \) = the mean of the distribution of study entry reticulocyte counts for CSSCD HbSS patients in the \( j \)th clinic, \( j \)th age group;

and \( S_j \) = the SD of distribution of study entry reticulocyte counts for CSSCD HbSS patients in the \( j \)th clinic, \( j \)th age group.

The above standardization was performed over 27 clinics and three age groups (two to nine years, ten to 19 years, \( \geq \) 20 years.)

Because the standardized values were difficult to interpret, mean reticulocyte values reported in Table 1 were calculated from patient values adjusted to the age-group mean for CSSCD HbSS patients from all clinics combined (age-adjusted reticulocyte count = \( \bar{X}_j + (X_{ik} - \bar{X}_j) \) where \( \bar{X}_j \) = the mean of the distribution of study entry reticulocyte counts for CSSCD HbSS patients in the \( j \)th age group).

Results are reported as mean \( \pm 1 \) SD.

*Cluster analysis: assays done on thrice-washed RBCs.
†Cytochemistry: assays done on cellulose-filtered RBCs. The assays and cytochemical studies were not done concurrently with the preceding group, and different reagent batches were employed.
‡Enzyme activity (IU/gHb).

Nondeficient all (100%) of those patients designated as nondeficient by the cluster classification.

Thirty-two males, not studied concurrently with this larger group, comprised those individuals who had leukocytes and platelets removed from their blood prior to enzyme assay and had cytochemical evaluation for detecting G6PD deficiency. The results of these studies are also shown in Table 2. Both G6PD-deficient patients had over 25% eluted cells, while GdA + and GdB individuals generally had less than 1% unstained cells. The leukocyte counts, initially 11.2 \( \pm \) 3.3 x 10^9/1, were reduced to less than 0.01 x 10^9/1 by filtration through the cellulose columns.

G6PD activity and HK activity were significantly elevated in GdA + and GdB males when compared to normal controls. This is consistent with the young mean-erythrocyte age in HbSS and has been previously reported. Figure 1A shows the distribution of G6PD and HK levels in GdA + and GdA - males. Contour plots for GdA + and GdA - males, which were constructed based on means, variances, and covariances of G6PD activity and hexokinase activity under the assumption that the distribution is normal are shown in Fig 1B. Of the 801 males, 10.4% were classified as G6PD deficient, while 18.4% had the catalytically normal polymorphic A + variant. There was no significant difference in enzyme activity when patients were age stratified (Table 3). The HK levels in 72 controls with GdB, taken from patients seen at the central laboratory, were similar to the mailed samples, indicating little deterioration in enzyme activity during the period of storage and shipping. In addition, when HK levels were assayed repetitively on a single blood sample over a two-week period, there was no fall in activity.

Age distribution of G6PD deficiency. Table 3 shows the

### Table 1. Laboratory Data of G6PD Deficient and Normal Patients with HbSS

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Hb(g/dL)*</th>
<th>MCV (fl)</th>
<th>Reticulocytes (%)</th>
<th>Bilirubin (mg/dL)</th>
<th>SGOT (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GdA +</td>
<td>92</td>
<td>8.1 ( \pm ) 1.2</td>
<td>89.7 ( \pm ) 8.5</td>
<td>13.3 ( \pm ) 6.1</td>
<td>3.6 ( \pm ) 2.2</td>
<td>50.0 ( \pm ) 27.9</td>
</tr>
<tr>
<td>GdA +</td>
<td>141</td>
<td>8.6 ( \pm ) 1.2</td>
<td>88.9 ( \pm ) 7.9</td>
<td>12.9 ( \pm ) 6.6</td>
<td>3.6 ( \pm ) 2.5</td>
<td>50.9 ( \pm ) 27.8</td>
</tr>
<tr>
<td>GdB</td>
<td>520</td>
<td>8.5 ( \pm ) 1.4</td>
<td>89.0 ( \pm ) 8.2</td>
<td>12.4 ( \pm ) 6.1</td>
<td>3.6 ( \pm ) 2.3</td>
<td>50.4 ( \pm ) 23.0</td>
</tr>
</tbody>
</table>

*Mean \( \pm \) 1 SD.
prevalence of G6PD deficiency in five different age groups. Using contingency table analyses (X^2) no significant difference was identified in the prevalence among the ages examined. This suggests that G6PD is not a selective factor for longevity or mortality in HbSS.

**Laboratory data.** Means and SDs for selected laboratory determinations are shown in Table 1. A multivariate analysis of variance model with effects for G6PD genotype, age group, and the three two-way interactions was fit to the hemoglobin level, mean corpuscular volume (MCV), and standardized reticulocyte data. The P value for G6PD genotype was 0.54. The same model was fit to the SGOT and bilirubin data. Only age group was identified as significant (P < .001). The P value for G6PD was .91.

**Clinical events.** The incidence of painful episode, sepsis, and acute anemic episodes in patients classified with G6PD deficiency was compared to those with normal activity using survival analysis. The time to the first episode from date of entry was defined as the time to failure. No significant differences were identified for any of the clinical events using either the log-rank or Gehan-Wilcoxin test statistics. Separate analyses were done for each outcome and for painful episode within each age group. The frequency of multiple painful episodes for the two G6PD groups was compared using the modified Mantel-Haenszel procedure controlling for length of follow-up. No differences were found between the two groups.
DISCUSSION

We sought answers to the following questions regarding the interaction of G6PD deficiency and HbSS. Is the prevalence of G6PD deficiency in HbSS different than that found in blacks with normal hemoglobin (HbAA) or sickle cell trait (HbAS), and does the prevalence change with age? Differences in prevalence might suggest that G6PD deficiency affects the survival of patients with HbSS. Does G6PD deficiency affect the degree of hemolysis in HbSS? Does G6PD deficiency increase the incidence of vaso-occlusive episodes, typified by painful crises, or increase the incidence of infection typified by sepsis? Are patients with G6PD deficiency and HbSS more likely to become acutely anemic than patients with HbSS alone? Because of the complexities of ascertaining the presence of G6PD deficiency in females,18,20 our analysis deals only with males.

The detection of G6PD deficiency in the presence of HbSS is complicated by the characteristics of the GdA− isozyme, the most prevalent variant causing G6PD deficiency in blacks. The instability of this enzyme leads to a rapid loss of activity, so that its levels are highest in the youngest red cells.21 The reticulocytosis of HbSS may therefore be responsible for nearly normal enzyme activity despite the presence of the GdA− gene.22,5 To control for the effects of cell age upon G6PD activity, we concurrently measured HK activity.7 This glycolytic enzyme is, like G6PD, cell-age dependent, and as HK deficiency is very rare, comparison of the activities of both enzymes permits the detection of individuals likely to have the GdA− gene.7 The enzyme levels we observed were similar to those reported by Bienzle et al6 and Beutler and coworkers.7 Our G6PD levels differ from those reported by Piomelli et al; however, these investigators did their assays under somewhat different conditions. In a subset of patients we evaluated the contribution of residual leukocytes and platelets to G6PD and HK activity and found little difference in activity when these cells were almost completely removed by filtration through cellulose columns when compared to the standard washing technique used in the bulk of our patients. In this same subsample we attempted to verify the accuracy of our G6PD genotype assignment by cluster analysis by using a cytochemical method for detecting deficient individuals.12 Although the numbers were small, G6PD activity in these patients was similar to that of the larger group. In addition, there was good concordance between the results of the cluster and discriminant analyses for classification of patients; however, 6% of G6PD-deficient individuals might have been misclassified as normals.

There appear to be some significant differences in the relative prevalence of the GdA− and GdA+ alleles reported in different studies. Bienzle et al6 found the prevalence of the GdA− and GdA+ alleles was similar in HbSS (16% and 21.6%) and in the general population of Ibadan, Nigeria (23% and 23.9%). Beutler and coworkers in California7 found that the GdA− allele was present in only 9% of controls but in 19.1% of HbSS patients and that the frequency of GdA− and GdA+ was similar in HbSS patients and their siblings with HbAA or HbAS. Yet the GdA+ frequency in HbAA controls was 24%. In an earlier report from Mississippi8 we found a GdA+ frequency of 22.7% in a small group of HbSS males, while the GdA− frequency was 4.5%. Piomelli and coworkers in New York22 have reported that 24% of HbSS males were GdA− and 13% GdA+, while in unrelated controls the frequency of both GdA− and GdA+ was 11%. In Jamaica 22% of 53 males with HbSS were G6PD deficient, and the prevalence in the general population was 10%.8 In the present study the GdA− frequency of 10.4% compares favorably with the frequency of GdA− in HbAA and HbAS males previously reported23 while about 18% of our group were GdA+. These discrepancies cannot easily be explained but may be a result of studying small numbers of patients,6,22 examining populations with different genetic backgrounds, and misclassification of GdA− and GdA+ individuals in HbSS. Cytochemical measurements of G6PD activity14,22 should make misclassifications less likely, and measurements of enzyme activity alone should suffice to distinguish GdA− from GdA+ in individuals without hemolytic anemia.7

While Piomelli and workers suggested that the high prevalence of G6PD deficiency in HbSS reflected a clinically beneficial effect of this interaction,8 Beutler et al,1 studying HbAA and HbAS male siblings of HbSS patients as controls, found similar frequencies of G6PD deficiency in both groups. They concluded that the frequency of G6PD deficiency paralleled that of other African genes. We found that the prevalence of the GdA− genotype was not significantly different when patients were stratified by age groups (Table 3). On the basis of our data it therefore seems unlikely that G6PD deficiency affects survival in males with HbSS.

There were no statistically significant differences noted in hemoglobin concentration, standardized reticulocyte count, MCV, SGOT, or bilirubin levels (Table 1). These measurements can reflect the intensity of hemolysis in HbSS. We made these laboratory measurements while patients were in a baseline condition and not in the midst of any acute events. It therefore remains possible that HbSS patients with G6PD deficiency may be more prone to the development of accelerated hemolysis when exposed to certain drugs or environmental influences that appear to provoke hemolysis in nonhemoglobinopathic patients with G6PD deficiency. This would be reflected in an increased incidence of acute anemic episodes in G6PD-deficient groups. We did not observe any differences in acute anemic episodes in these patients; however, the bulk of these episodes were aplastic crises or a result of unknown causes, and unequivocal accelerated hemolysis seemed quite rare. Also we did not note that painful episodes, or the occurrence of sepsis, were more prevalent in G6PD-deficient HbSS patients. Gibbs and coworkers have reported similar results from their study of Jamaican patients.8

A number of earlier studies have shown an increased prevalence of G6PD deficiency in HbSS.1,2,7 The interpretation of these data is complicated by the use of methods for detection of G6PD deficiency that is unreliable in patients with HbSS1 or by small sample size.2,7 Reports of accelerated hemolysis when HbSS and G6PD deficiency coincide are anecdotal, and such an association is not borne out by our observations.2

The questions we posed initially can be answered nega-
We find little influence of G6PD deficiency upon HbSS in males. Specifically, there is no evidence that G6PD deficiency enhances survival, intensifies hemolysis, or increases the incidence of acute anemic episodes or sepsis in HbSS.

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REFERENCES

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