Low Proportions of Glycosylated Hemoglobin Associated with Hemoglobin S and Hemoglobin C

H. Aleyassine

Using a cation-exchange chromatographic method, we found normal or subnormal values for glycosylated hemoglobin in a few diabetic patients with persistent hyperglycemia. Subsequent investigations revealed that these unexpected results had originated from black patients with diabetes. In view of common occurrence of abnormal hemoglobins in the Negro population, we subjected blood preparations to electrophoresis on cellulose acetate and acrylamide gel. The results have shown the presence of hemoglobin S or hemoglobin C in each patient. When allowance was made for the percentage of the abnormal hemoglobin, the "corrected values" of glycosylated hemoglobin increased to the diabetic range. Furthermore, the corrected values agreed well with the "expected values" calculated from a regression line correlating fasting blood glucose concentrations and proportions of glycosylated hemoglobin in more than 300 diabetics with no evidence of hemoglobinopathy. We conclude that in diabetic patients presenting with hemoglobin S or hemoglobin C, there is a considerable decrease in the values for glycosylated hemoglobin as measured by cation-exchange chromatographic methods, and that this decrease is proportional to the percentage of the abnormal hemoglobin.

Additional Keyphrases: diabetes · hemoglobin variants

Recent studies have shown that percentages of glycosylated hemoglobin (glycosylated Hb) are increased in diabetic patients (1, 2) and that there is a positive correlation between the degree of hyperglycemia and the proportion of glycosylated Hb (3–7). Because glycosylation of hemoglobin is a slow and continuous process that takes place during the entire life of the erythrocyte (7), it has been proposed (5) that glycosylated Hb values be used as a long-term indicator of diabetic control.

Using a cation-exchange chromatographic method, we have confirmed these observations in a large number of ambulatory diabetic subjects. However, in a small proportion of hyperglycemic patients we have consistently found normal or subnormal values for glycosylated Hb. Subsequent investigations revealed that these unexpected results originated from black diabetic patients. In view of common occurrence of abnormal hemoglobin in the Negro population, we studied the possible relationship between hemoglobinopathies and low glycosylated hemoglobin.

Materials and Methods

All patients were from the Diabetic Center of the Montreal General Hospital. Normoglycemic subjects with no clinical evidence of diabetes served as the control. Plasma glucose was measured with the Beckman Glucose Analyzer, with use of a glucose oxidase reagent. For glycosylated hemoglobin, blood specimens were drawn into a collection tube with EDTA anticoagulant and tested within 2 h. Glycosylated Hb was separated from "other hemoglobins" by the column-chromatographic method of Isolab (Akron, OH 44321). The percentage of the fast component was calculated from readings of the absorbances of the fast and of the total hemoglobin at 415 nm with a Gilford spectrophotometer. Abnormal values reported in this study were also tested, for confirmation, by the Glycosylated Hemoglobin Quik Column method (Helena Labs., Beaumont, TX 77704). The methods (Isolab and Hel-
Table 1. Values for Glucose, Abnormal Hemoglobin, and Glycosylated Hemoglobin in Six Negro Patients\(^a\)

<table>
<thead>
<tr>
<th>Abnormal Hb (% of total Hb)</th>
<th>Plasma glucose, mg/dL, during fasting</th>
<th>Values for glycosylated Hb (% of total Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb S (38.9)</td>
<td>1.57</td>
<td>Obtained</td>
</tr>
<tr>
<td>Hb C (43.5)</td>
<td>1.41</td>
<td>Corrected</td>
</tr>
<tr>
<td>Hb S (39.1)</td>
<td>3.22</td>
<td>Expected</td>
</tr>
<tr>
<td>Hb S (40.6)</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>Hb C (41.2)</td>
<td>2.28</td>
<td></td>
</tr>
<tr>
<td>Hb S (39.6)</td>
<td>2.65</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The percentage of abnormal hemoglobins was determined spectrophotometrically, after hemoglobin fractionation by acrylamide gel electrophoresis. "Obtained" values refer to glycosylated Hb values obtained by column chromatography. "Corrected" values were calculated using the following formula:

\[
\text{corrected value} = \frac{\text{obtained value} \times 100}{(100 - \text{percentage of abnormal Hb})}
\]

"Expected" values were determined as follows: a regression line correlating blood glucose during fasting and glycosylated Hb values was constructed for results from more than 300 diabetic patients with no evidence of hemoglobinopathy. The expected glycosylated Hb values (dependent variable) of patients with hemoglobinopathy were read from this regression line, with blood glucose concentration from fasting patients as the independent variable.

Results

When a blood hemolysate is chromatographed according to the cation-exchange chromatographic method of Isolab, the fast component of hemoglobin (glycosylated Hb) emerges from the column with the first eluant. The remaining hemoglobins, which are retained by the resin, migrate diffusely over a distance of 1.5–2 cm from the top of the column. However, in some Negro subjects we have found a distinctive chromatographic pattern characterized by the presence of a dense layer of hemoglobin at the top of the column. This characteristic pattern was invariably associated with a decreased proportion of glycosylated hemoglobin.

Because of the frequent occurrence of hemoglobinopathy in Negro subjects, preparations of blood specimens with abnormal chromatographic patterns were subjected to electrophoresis on cellulose acetate or acrylamide gel, to detect any abnormal hemoglobin. The presence of Hb S or Hb C was demonstrated in all such samples.

To investigate the possible relationship between hemoglobinopathy and the low values for glycosylated Hb, we determined the percentages of abnormal hemoglobins in six diabetic Negro subjects in whom the presence of Hb S or Hb C was already established. As can be seen from Table 1, in spite of fasting hyperglycemia, glycosylated Hb values obtained by the column-chromatographic method ("obtained values") were subnormal in two patients and within the normal range in the four others (normal range: 6.5–8.8%)

However, when allowance was made for the percentage of abnormal hemoglobins, the "corrected values" increased to the diabetic range.

We also calculated the expected glycosylated Hb values corresponding to the fasting blood glucose concentration in each of these six patients, from a regression line correlating glycosylated Hb and fasting blood glucose concentrations in more than 300 diabetics with no evidence of hemoglobinopathy. The "expected values" agreed well with the "corrected values" (Table 1).

It is therefore clear that in the diabetic patients presenting with Hb S or Hb C, there is a considerable decrease in glycosylated Hb as measured by cation-exchange chromatographic methods, and that this decrease is proportional to the percentage of the abnormal hemoglobin.

Discussion

Glycosylated Hb has been shown to be the result of binding of a carbohydrate moiety to the N-terminals of the \( \beta \) chains of hemoglobin A \((8, 9)\). Thus it is conceivable that any alteration in the structure of the \( \beta \)-chain could result in interference with the glycosylation process. In the present studies, we have shown that the presence of Hb S and Hb C—two hemoglobins with altered \( \beta \)-chains—was associated with a marked decrease in measurable glycosylated Hb. Two different explanations may be offered for this phenomenon. In the first instance, it is possible that, unlike hemoglobin A, Hb S and Hb C are incapable of undergoing glycosylation, and this alone accounts for the low glycosylated Hb observed in the two types of hemoglobinopathies. The second possibility is that Hb S and Hb C are normally subjected to glycosylation but are not eluted from the column by the "fast" buffer solution, resulting in these apparent low proportions of glycosylated Hb.

Whatever the exact reason for this observation, the fact remains that in a significant number of diabetic Negro patients the measured glycosylated Hb values should be corrected by prior determination of abnormal hemoglobin. When this is done, there is a close agreement between the "corrected values" as determined by acrylamide gel electrophoresis and "expected values" as calculated from the regression line.

It has been reported \((4, 6)\), and confirmed by our own studies, that there is a positive correlation between the concentrations of blood glucose during fasting and values for glycosylated Hb. In a study of more than 300 diabetics, we have found (unpublished results) that the correlation coefficient \((r)\) between these two variables varies according to the mode of therapy. The highest \(r\) (0.78) was found in patients treated with diet alone, and the lowest (0.54) in those treated with insulin. In the group of six black diabetics reported here, four patients were being treated with diet alone, one with an oral hypoglycemic agent, and one with insulin injections. Therefore, the expected values for glycosylated Hb in each patient were determined from the regression line corresponding to the patient's mode of therapy. This increased the precision of the results.

Finally, we have found that an interesting feature of the cation-exchange chromatographic procedure of Isolab used...
in this study was its potential for rapid screening of Hb S and Hb C. However, the procedure cannot differentiate between the two abnormal hemoglobins and therefore other, more specific methods such as electrophoresis are required for further identification.

Although the present study was limited to two of the most common abnormal hemoglobins, other forms of hemoglobinopathies possibly may also exert a similar influence on glycosylated Hb. In addition, our results indicate that all cases of unexplained low glycosylated Hb values should be investigated for the presence of abnormal hemoglobins.

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References