Four Assay Methods for Glycated Hemoglobin Compared as Screening Tests for Diabetes Mellitus: The Islington Diabetes Survey

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We assessed the utility of four methods of glycated hemoglobin assay (agar gel electrophoresis with Schiff base, agar gel electrophoresis with prior removal of Schiff base, boronate affinity chromatography, and ioelectric focusing) as screening tests for diabetes mellitus, studying 223 subjects undergoing an oral glucose-tolerance test after fasting and 2 h after ingestion of 75 g of glucose. Assessment of glucose tolerance status according to the 1980 World Health Organization diagnostic criteria indicated that 13 subjects (5.8%) had diabetes, 48 (21.5%) had impaired glucose tolerance, and nine (4.0%) could not be classified (six of these because of missing values). Use of receiver–operating characteristic curves to compare the assays as screening tests showed the affinity chromatography assay to be superior. Assays for glycated hemoglobin after fasting had better precision than post-glucose-load assays as screening tests, and test characteristics of glycated hemoglobin assays on fasting subjects were similar to those of the blood-glucose estimation after fasting. We conclude that measurement of glycated hemoglobin may be a useful screening test for diabetes, and that such measurement of stable glycated hemoglobin is as accurate as measurement of fasting blood-glucose in screening for diabetes. For this use, we found methods that measure stable glycated hemoglobin superior to that measuring both stable and labile Schiff-base fractions.

Additional Keyphrases: chromatography, affinity · electrophoresis, agar gel · isoelectric focusing · receiver–operating characteristic curves · glucose-tolerance test

Diabetes mellitus causes increased morbidity and mortality, a considerable part of which may be preventable. In the community at large, only half of all patients with diabetes mellitus have been diagnozed (1, 2). Screening for diabetes may be an important means of identifying groups at high risk of debilitating illness or early death. However, screening is hampered by the lack of a single sensitive and specific screening test. The World Health Organization (WHO) has recommended the 75-g oral glucose tolerance test as the standard diagnostic test for diabetes (3) and has endorsed the use of a single measurement of blood glucose 2 h after a glucose load in studies intended to assess the prevalence of diabetes (4). In both of these methods subjects must undergo a glucose-tolerance test that takes at least 2 h to perform.

The Islington Diabetes Survey was designed to evaluate glycated hemoglobin assays as screening tests for diabetes in a population older than 40 years, randomly selected from the age–sex register of a single North London group practice (2, 5). We have previously reported a study of the characteristics of an assay of glycated hemoglobin as a screening test for diabetes mellitus (6). In that study we used an agar gel electrophoretic method to measure both stable and labile (Schiff base) fractions of glycated hemoglobin. Other assay methods suggested to have superior characteristics as screening tests have also distinguished normal subjects from those with impaired glucose tolerance (7). We have therefore compared four different methods of assay for glycated hemoglobin as screening tests in 214 subjects recalled in the second phase of a community study of 1084 subjects in North London. In this recall population, the prevalence of impaired glucose tolerance (IGT) and diabetes, defined according to 1980 WHO criteria (3), was 21.5% and 5.8%, respectively (2).

Materials and Methods

In the screening phase of the Islington Diabetes Survey, the glucose-tolerance status of the subjects was assessed by a single determination of the concentration of glucose in capillary blood 2 h after a 75-g oral glucose load (2hBG). In the recall phase, a stratified sample of 223 subjects selected from among those screened, biased towards those with higher results for 2hBG and glycated hemoglobin, underwent a full 75-g oral glucose-tolerance test (GTT). Of these, 13 subjects (5.8%) were confirmed to have diabetes and 48 (21.5%) to have IGT. In this latter testing, glycated hemoglobin was measured both in fasted subjects and 2 h after the glucose load by four methods:

- Agar gel electrophoresis (Corning Medical Ltd., Halstead, Essex, U.K.), measuring glycated hemoglobin plus the labile Schiff-base fraction (TotalGHb). Within- and between-assay coefficients of variation (CVs) were respectively 3.9% and 6.2% in the nondiabetic range (TotalGHb <8.0%) and 2.0% and 1.7% in the diabetic range (TotalGHb ≥8.0%).

- Agar gel electrophoresis of hemolysate after incubating it in dipotassium phthalate to remove the Schiff-base fraction (8) (StableGHb). Within- and between-assay CVs were respectively 3.9% and 6.2% in the nondiabetic range and 2.0% and 1.7% in the diabetic range.

- Boronate affinity chromatography, in columns containing 1 mL of affinity gel (Pierce & Warriner, Chester, Cheshire, U.K.) (9). Within- and between-assay CVs were respectively 2.0% and 1.8% in the nondiabetic range and 1.5% and 1.5% in the diabetic range.

- Isoelectric focusing (10). Within- and between-assay CVs were respectively 4.9% and 5.3% in the nondiabetic range and 2.0% and 3.6% in the diabetic range.

Statistical methods: Test characteristics—sensitivity, specificity, and the predictive value of a positive test result

1 Nonstandard abbreviations: IGT, impaired glucose tolerance; GTT, glucose tolerance test; 2hBG, concentration of blood glucose 2 h after oral glucose load; GHb, glycated hemoglobin, including (Total) or excluding (Stable) the labile Schiff-base fraction; ROC, receiver–operating characteristic; and PV+, predictive value of a positive test result.

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(PV+) (11)—were calculated over a range of cutoff points and the accuracy of each assay was compared by use of receiver-operating characteristic (ROC) curves, in which true-positive fraction (sensitivity) is plotted against false-positive fraction (1 - specificity) (12).

To compare groups of data, we used either paired or unpaired two-tailed t-tests. Results are expressed as mean ± SD. Test characteristics are quoted with 95% confidence-interval ranges.

Results

Assessable GTT results were obtained in 214 of the recall-phase 223 subjects (96.0%). Thirteen (5.8%) subjects had diabetes, 48 (21.5%) had impaired glucose tolerance, 153 (68.6%) had normal tolerance, and nine (4.0%) could not be classified. In six of these nine, failure to classify was because of missing blood-glucose values; one subject with a high value for fasting blood-glucose concentration had normal 1-

h and 2-h values, and two subjects had isolated instances of increased 2-h values.

Values for glycated hemoglobin after fasting, distributed according to glucose-tolerance status, as measured by each of the four methods, show considerable overlap between groups of subjects with normal tolerance, impaired glucose tolerance, and diabetes (Figure 1). The distribution of post-load values for glycated hemoglobin shows a similar degree of overlap. However, the proportions of glycated hemoglobin were substantially higher after the load when assayed by the TotalGHb method. In the other methods, which measure only stable glycated hemoglobin, there were no significant differences between fasting and post-load measurements of glycated hemoglobin as assessed by paired Student's t-test.

Table 1 compares the specificity and PV+ of the assays at a fixed sensitivity of 90%. These characteristics are also compared by use of ROC curves in Figure 2. The confidence intervals for both sensitivity and specificity for the assays.

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**Fig. 1.** Distribution of fasting (top) or post-load (bottom) glycated hemoglobin assayed by (a) agar gel electrophoresis including labile Schiff base fraction (TotalGHb), (b) agar-gel electrophoresis excluding labile Schiff-base fraction (StableGHb), (c) affinity chromatography (AC), and (d) isoelectric focusing (IEF) according to glucose-tolerance status.

DM, diabetes mellitus; no. of subjects is also shown.

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Table 1. Specificity and Predictive Value of a Positive Test Result (PV+) for Fasting Blood Glucose and Fasting Glycated Hemoglobin Assays as Screening Tests for Diabetes Mellitus at 90% Sensitivity

<table>
<thead>
<tr>
<th>Assay</th>
<th>Specificity</th>
<th>PV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood-glucose</td>
<td>93.6 (89.8-100)</td>
<td>45.5 (27.8-63.2)</td>
</tr>
<tr>
<td>Total GHb</td>
<td>59.3 (52.4-66.2)</td>
<td>12.6 (7.1-18.1)</td>
</tr>
<tr>
<td>Stable GHb</td>
<td>86.0 (79.5-92.5)</td>
<td>34.5 (17.6-51.4)</td>
</tr>
<tr>
<td>Affinity chromatography</td>
<td>90.9 (86.9-94.9)</td>
<td>39.4 (21.9-56.9)</td>
</tr>
<tr>
<td>Isoelectric focusing</td>
<td>74.0 (61.8-86.2)</td>
<td>25.7 (5.4-45.9)</td>
</tr>
</tbody>
</table>

In an earlier report, we compared the accuracy of the assay of glycated hemoglobin that includes both labile and stable fractions (TotalGHb) with that of a single post-load value for blood-glucose, or a value after fasting, as a screening test for diabetes and found it to be less accurate than either (6). Results of our present study suggest that this may be a feature of the assay method employed, because the accuracy of the methods that measure only the stable glycated-hemoglobin fraction was found to be similar to that of blood glucose measured after fasting. Although it would be expected that subjects with impaired glucose tolerance or diabetes would have higher concentrations of Schiff base, especially after a glucose load (17), the presence of the labile Schiff base paradoxically does not improve assay characteristics. The assay of blood glucose after fasting was more nearly accurate (although, as indicated in Table 1, not significantly so) as a screening test than were any of the post-load assays of glycated hemoglobin. Figure 1 shows that the affinity chromatography assay may better distinguish subjects with impaired glucose tolerance from normal individuals than the other assays do. About a third (12/46 and 18/45, respectively) of subjects with impaired glucose tolerance had values by affinity chromatography >8.0% (our upper normal limit), whereas the distribution of glycated hemoglobin values for subjects with impaired glucose tolerance assayed by the other methods overlapped those of normal subjects more exactly. The ROC analysis shows the affinity chromatography assay to have better precision than the other assay methods studied, although the confidence-interval ranges are wide and a larger study would be necessary to establish this with assurance.

Neither the test characteristics nor the accuracy of the assays was improved by taking the mean of the values for fasting and post-load glycated hemoglobin in each subject in order to minimize the effect of assay variability (5). Nor were they improved by defining test characteristics on those subjects whose glucose-tolerance status remained unchanged between screening and recall examinations (5).

In detecting subjects with any degree of glucose intolerance (both impaired glucose tolerance and diabetes) the confidence-interval ranges for the fasting assays and for all the post-load assays overlap considerably, indicating that no assay method was significantly superior. ROC curves showed poor accuracy for all assays as screening tests for any degree of glucose intolerance (5).

Several studies have reported the use of the assay of glycated hemoglobin as a screening (18-23) or diagnostic (7, 24-29) test for diabetes. The screening studies have shown the assay to have much poorer characteristics than either the fasting blood glucose or a single 2hBG, though a high specificity has been reported in several studies (18, 19, 22, 29). With a single exception (29), these workers used an assay method that included the labile Schiff-base fraction. Studies that measured only stable glycated hemoglobin in subjects referred to hospital for GTT because of suspected glucose intolerance showed better characteristics (7, 24, 25), but this is likely to be ascribable to subject selection rather than assay method, because such subjects are more likely to have hyperglycemia after fasting and consequently an increased value for glycated hemoglobin (30). One small study (27), in which the measurement of HB A1 was compared with that of Hb A1c in screening for diabetes, found that the measurement of total Hb A1 (i.e., both labile and stable fractions) showed superior test characteristics (27).

The previously reported studies comparing the perform-
ance of blood glucose estimations with assays of glycated hemoglobin have compared single levels of sensitivity and specificity for each assay method, making difficult any direct comparison of their results (5). Recently, ROC analysis was used to re-assess the data presented in a study of the efficacy of glycated hemoglobin as a screening test for gestational diabetes, with different results from those of the original authors (31).

We conclude that assays that measure only stable glycated hemoglobin are more nearly accurate than that including the labile Schiff-base fraction as screening tests for diabetes, and that the affinity chromatography assay is more nearly accurate than the other methods tested. This study also indicates that measurement of glycated hemoglobin may be an acceptable screening test for diabetes mellitus.

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References