Utility of Glycated Hemoglobin in Diagnosing Type 2 Diabetes Mellitus: A Community-Based Study

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Context: Although glycated hemoglobin (HbA1c) has recently been incorporated as a diagnostic test by the American Diabetes Association, its validity needs to be established in Asian Indians in a community setting.

Objective: The objective of the study was to assess the validity of HbA1c as a screening and diagnostic test in individuals with newly detected diabetes mellitus.

Design and Setting: Community based randomized cross sectional study in urban Chandigarh, a city in north India, from April 2008 to August 2009.

Subjects: Subjects included 1972 subjects aged 20 yr or older.

Intervention: Intervention included an oral glucose tolerance test and glycated hemoglobin in all the subjects.

Main Outcome Measures: Utility of HbA1c as a diagnostic method in newly detected diabetes mellitus subjects was evaluated.

Results: Using World Health Organization criteria for diagnosis of diabetes mellitus, 134 (6.7%) had newly detected diabetes mellitus, 192 (9.7%) known diabetes mellitus, 329 (16.6%) prediabetes, and 1317 (69.4%) were normal of 1972 people screened. Using only the ADA criteria, 38% people were underdiagnosed. An HbA1c level of 6.1% had an optimal sensitivity and specificity of 81% for diagnosing diabetes. A HbA1c level of 6.5% ($\pm$2 SD) and 7% ($\pm$2.7 SD) had sensitivity and specificity of 65 and 88% and 42 and 92%, respectively, with corresponding positive predictive value and negative predictive value of 75.2 and 96.5% and 90.4and 94.4%, respectively, for diagnosis of newly detected diabetes mellitus.

Conclusion: A HbA1c cut point of 6.1% has an optimal sensitivity and specificity of 81% and can be used as a screening test, and a cut point of 6.5% has optimal specificity of 88% for diagnosis of diabetes. **(*J Clin Endocrinol Metab* 95: 2832–2835, 2010)**

The diagnosis of diabetes mellitus is classically based on blood glucose levels either fasting or 2-h plasma glucose (2hPG) after an oral glucose tolerance test (OGTT) using 75 g anhydrous glucose. The diagnostic criteria of the American Diabetes Association (ADA; 1997) and World Health Organization (WHO; 1999) are essentially the same and include either fasting plasma glucose (FPG) 126 mg/dl (7 mmol/liter) or greater or 2hPG 200 mg/dl (11.1 mmol/liter) or greater (1, 2). These tests need to be confirmed on a subsequent day in the absence of unequivocal hyperglycemia. Recently the ADA recommended glycosylated hemoglobin (HbA1c) as a diagnostic test. A HbA1c level of 6.5% or more

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Abbreviations: ADA, American Diabetes Association; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; 2hPG, 2-h plasma glucose; KDM, known diabetes mellitus; NDM, newly detected diabetes mellitus; NPV, negative predictive value; OGTT, oral glucose tolerance test; PPV, positive predictive value; ROC, receiver-operating characteristic; WHO, World Health Organization.

(to be reconfirmed on a subsequent day) has been introduced for diagnosis of diabetes. The problems with blood glucose estimations include high intraindividual biological variability (4–14%), preanalytical variability like the method of collection, storage (rate of fall of glucose in samples at room temperature 3–8 mg/dl · h) and lifestyle measures like exercise and calorie restriction and difficulty in ensuring fasting state before blood glucose measurement (3, 4). HbA1c overcomes many of these difficulties as fasting state is not required and analytical variability is less than 2% and gives glycemic status over the past 2–3 months. The main disadvantages are it is costly, requires National Glycohemoglobin Standardization Program-certified HPLC method to estimate the HbA1c. It is also affected by hemoglobinopathies, recent hemolysis, high triglyceride levels, and some of the drugs like salicylates and vitamin C and E. This study was carried out as part of a large epidemiological survey on prevalence of diabetes mellitus in Chandigarh.

Subjects and Methods

The present study was part of a large cross-sectional study on prevalence of diabetes mellitus among adults in Chandigarh, a city with the highest per-capita income and literacy rate in India. The Union Territory of Chandigarh is a well-planned city with 56 sectors and subsectors. Six sectors were picked by simple randomization, and in each sector all the adults 20 yr of age or older were screened for diabetes mellitus based on standard OGTT using 75 g anhydrous glucose (details are described elsewhere). The FPG and 2hPG was estimated using a glucometer (Ultra 2; Johnson and Johnson, New Brunswick, NJ), which was validated. In every 10th case, venous plasma glucose was estimated by using the glucose oxidase method (Autoanlayzer 902; Hitachi, Tokyo, Japan). The correlation coefficients for FPG and 2hPG by glucometer and laboratory methods were 0.94 and 0.81. The diagnosis of diabetes mellitus was based on WHO 1999 criteria. Blood for HbA1c was collected in EDTA vials and estimated by Bio-Rad 10 system (Bio Rad, Hercules, CA) functioning on HPLC-based ion exchange chromatography. The Bio-Rad machine was conforming to National Glycohemoglobin Standardization Program standardized to the Diabetes Control and Complications Trial. The intraassay coefficients of variation for normal and diabetic subjects were 0.81 and 0.48%. The interassay coefficients of variation for normal and diabetic patients were 2.35 and 1.65%.

Detailed anthropometry (height, weight, and waist circumference) and blood pressure were taken using standard methods. Sensitivity, which is the proportion of subjects correctly identified as diseased (true positives), and specificity, which is the true negative subjects identified as nondiseased, form the basis for analysis of the present study.

Statistical analysis

Sensitivity and specificity at various cutoff levels of HbA1c were calculated and receiver-operating characteristic (ROC) curves were constructed for study population excluding known diabetes mellitus subjects.

TABLE 1. Distribution of HbA1c in various subjects

Character	Number	HbA1c Mean ± SD	95% CI
Normal	1317	5.44 ± 0.56	5.42–5.48
KDM	192	8.42 ± 2.21	8.10–8.73
NDM	134	7.43 ± 2.09	7.08–7.78
Prediabetes	329	5.90 ± 0.66	5.83–5.97
IFG	125	5.76 ± 0.55	5.67–5.86
IGT	141	5.98 ± 0.72	5.86–6.10
IFG + IGT	63	6.01 ± 0.69	5.84–6.19

CI, Confidence interval; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

Results

In all, 2368 people were approached in six sectors for the study purpose, of which 123 people were nonresponders (response rate 94%). Of these, HbA1c analysis was available in 1972 subjects. The subjects in whom HbA1c values were not available (n = 255) were similar in respect to baseline characteristics and distribution of normal and diabetic subjects. Three hundred twenty-six people (16.4%) were detected to have diabetes, which included 192 with known diabetes (9.7%) and 134 with newly detected diabetes (6.7%). The prediabetes group constituted 16% of the study population. People with known diabetes mellitus (KDM) were already on treatment with medications, so the diagnostic utility of HbA1c was assessed in newly detected diabetes mellitus (NDM) subjects only. The mean age of NDM subjects was 53.6 ± 13.9 yr and body mass index was 27.3 ± 4.7 kg/m^2. The sensitivity and specificity of FPG for diagnosis of NDM at 7 mmol/liter (ADA cutoff) were 58 and 93%, respectively. Taking ADA criteria based on FPG levels alone would have missed diabetes in 51 subjects (38%).

The mean HbA1c of healthy subjects was 5.44 ± 0.56% with 95% confidence interval levels ranging from 5.42 to 5.48%. The mean HbA1c levels in NDM, KDM, and prediabetes were 7.43 ± 2.09, 8.42 ± 2.21, and 5.9 ± 0.6, respectively (Table 1). ROC curves were constructed for normal and NDM people (Fig. 1). The sensitivity and specificity of HbA1c at various cutoff levels of NDM subjects are shown in Table 2. HbA1c levels of 6.5 and 7%, which are 2 SD and 2.7 SD above the mean in healthy subjects, had sensitivity and specificity of 65 and 88% and 42 and 92%, respectively. The positive predictive value (PPV) and negative predictive value (NPV) at HbA1c levels of 6.5 and 7% were 75.2 and 96.5% and 90.4 and 94.4%, respectively.

Further subanalysis of 51 subjects who had FPG less than 126 mg/dl and whose 2hPG was diagnostic (subjects missed based on ADA FPG criteria alone), the sensitivity and specificity of HbA1c at various cutoff levels of 6.1, 6.5, and 7% for diagnosing diabetes by OGTT were 88 and 30%, 78 and 55%, and 57 and 83%, respectively (results not shown).

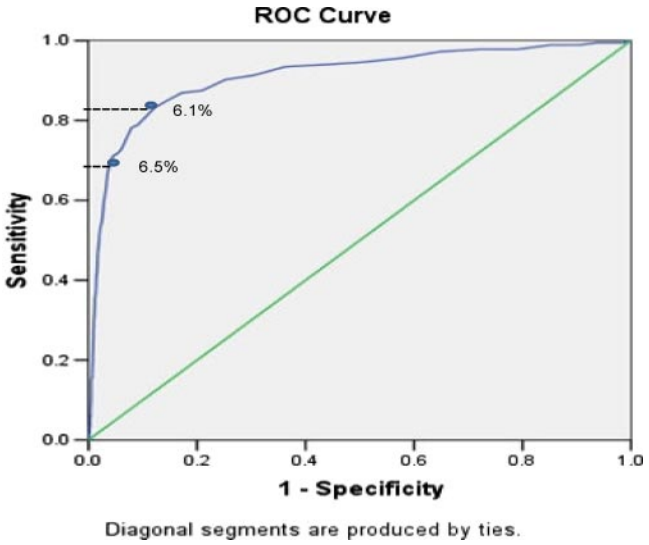

FIG. 1. ROC curve of HbA1c in NDM subjects.

Discussion

In the present study, the prevalence of diabetes based on WHO 1999 criteria using gold standard OGTT was 16.4%; with known diabetes, 9.7%; and new diabetes, 6.7%. A HbA1c level of 6.5% has an optimum specificity of 88% and NPV of 96.5% to be considered as a diagnostic test for diabetes mellitus.

The ADA recommends the diagnosis of diabetes mellitus based on FPG levels both in individuals and also community-based studies or HbA1c level of 6.5% or more. In the present study, diagnosis of diabetes based only on ADA criteria would have missed 38% of subjects with diabetes. Similar observations were found in various studies (5, 6). In the Early Diabetes Intervention Program study, 24 and 50% of subjects with OGTT-confirmed diabetes had FPG levels between 5.5 and 6.0 and 6.1–6.9 mmol/liter, respectively (5).

The mean HbA1c in 1317 healthy subjects was 5.44 ± 0.56%. By taking a HbA1c cutoff level of 6.1%, which is 1.2 SD above the mean in normal subjects, the sensitivity and specificity were 81% for diagnosis of NDM. The sensitivity remained high in those diagnosed to have diabetes by FPG of 126 mg/dl or greater, 2hPG of 220 mg/dl or greater, or both of −87, 88, and 81%, respectively. These results are similar to a large metaanalysis by Bennett *et al.* (7), who reported that a HbA1c level of 6.1% had a sensitivity and specificity of 78–81% and 79–84%, respectively. Similar to the present study, Ko *et al.* (6) reported a sensitivity and specificity of 77.5 and 78.8% at a HbA1c level of 6.1%. However different studies reported different sensitivities and specificities at various HbA1c levels possibly due to different ethnicity and assay methods adopted as described previously (8). Further subanalysis of subjects with FPG between 100 and 125 mg/dl, the same cutoff point (6.1%) has a sensitivity and specificity of 68 and 13% (results not shown). So use of HbA1c in subjects with FPG level less than 126 mg/dl has reasonable sensitivity but poor specificity. These results are similar to Perry *et al.* (5), who reported the increased sensitivity of HbA1c at 6.1% in subjects with FPG levels between 100 and 125 mg/dl who were diagnosed by 2 h OGTT. The sensitivity increased from 45 to 61% when compared with FPG criteria only.

TABLE 2. Sensitivity and specificity of HbA1c at various cutoff levels in NDM subjects

HbA1c cutoff level (%)	Sensitivity (%)	Specificity (%)
5.7	92	63
5.8	92	68
6.0	83	77
6.1	81	81
6.2	76	84
6.3	73	86
6.4	70	87
6.5	65	88
6.6	62	89
6.9	47	91
7.0	42	92

Four community-based studies looked into the role of HbA1c as a diagnostic test by taking the OGTT as the standard for diagnosis of diabetes (9–12). Similarly hospital-based studies reported different sensitivity and specificity at different HbA1c levels (13–16). In the AusDiab study, Colagiuri *et al.* (9) studied the usefulness of HbA1c as a diagnostic test in 10,447 Australian subjects with one or more risk factors. They found a sensitivity and specificity of 78.7 and 82.8% at a HbA1c level of 5.3%. This is in contrast to the present study, which has high sensitivity of 96% but low specificity of 35% at similar HbA1c level. These differences may possibly be due to lesser number of subjects in the present study, although it is statistically more feasible. An optimal cutoff for screening people without known diabetes appears to be a HbA1c level of 6.1%, which has a reasonable sensitivity and specificity. Based on the present study and studies by others, a HbA1c level of 6.1% is ideally suited as a screening tool.

In the present study, a HbA1c level of 6.5% has a sensitivity and specificity of 65 and 88% with a PPV and NPV of 75.2 and 96.5%, respectively. By taking a cutoff of 7%, the specificity increased to 92% and sensitivity decreased to 42% with a PPV and NPV of 90.4 and 94.4%, respectively. For a disease like diabetes, which is widely prevalent in the community, one needs a test with good specificity to prevent large false positives to cause alarm. The specificities of 81 and 88% at HbA1c cutoff levels of 6.1 and 6.5% are still suboptimal. For diagnostic purposes, a cutoff level of 6.5% was recommended by Saudek *et al.* in 2008 (17). The cutoff level of 6.5% was based on 3 SD

above the mean HbA1c level in the National Health and Nutrition Examination Survey III study (5.17 ± 0.45), which had sensitivity and specificity of 43 and 99.6%, respectively. They also proposed a repeat HbA1c in subjects with HbA1c level of 7% or more to confirm the diagnosis (repeat HbA1c ≥6.5%). However, for persons with HbA1c level between 6.5 and 6.9%, the diagnosis is based on either FPG or random plasma glucose of 200 mg/dl or greater. An international expert committee report on HbA1c as a diagnostic test also chose 6.5% as the cutoff for diagnosis of diabetes based on the study results of The Evaluation of Screening and Early Detection Strategies for Type 2 Diabetes and Impaired Glucose Tolerance (DETECT-2), which is based on moderate retinopathy. Moderate retinopathy was almost nonexistent in subjects with HbA1c level less than 6.5% and progressively increases above this level (18). Although the ADA 2010 guideline recommends a HbA1c level of 6.5% for diagnosis of diabetes, it did not specify its sensitivity and specificity. However, it mentions that a HbA1c cut point of 5.7% has a sensitivity of 39% and a specificity of 91% to identify impaired fasting glucose. The present study clearly showed a specificity of 88% at HbA1c cutoff level of 6.5% for diagnosing diabetes and further validates ADA recommendations (19).

Conclusion

The present study shows that a HbA1c level of 6.1% has optimal sensitivity and specificity to be considered as a screening test. A HbA1c level of 6.5% has reasonably good specificity for diagnosis of diabetes and is in complete concordance with ADA recommendations.

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