Introduction

Diabetes mellitus, especially type 2 diabetes, is a burgeoning health problem and is a significant contributor to noncommunicable diseases-related morbidity and mortality worldwide. A significant proportion of diabetes cases still remain undiagnosed in India. Plasma glucose (PG) tests have well-established role in the diagnosis of diabetes and prediabetes. The American Diabetes Association (ADA) recommended glycosylated hemoglobin (HbA1c) values of ≥6.5% (≥48 mmol/mol as per the International Federation of Clinical Chemistry and Laboratory Medicine) for diabetes diagnosis and 5.7%–6.4% (39–46 mmol/mol) for identifying high-risk individuals for the future diabetes (i.e., prediabetes).

Abstract

Background: Glycosylated hemoglobin (HbA1c) has not been evaluated extensively for diabetes and prediabetes diagnosis and short-term variability of fasting plasma glucose (FPG), 2-h PG post-75 g glucose load (2 hPG) and HbA1c has not been studied among Indians. Objectives: The study aimed to compare the sensitivity of HbA1c, FPG and 2 hPG for diabetes and prediabetes diagnosis as per the American Diabetes Association criteria, assess short-term variability of three tests and determine optimal HbA1c cutoffs for diabetes and prediabetes diagnosis among high-risk South Indians. Methods: This diagnostic accuracy study, conducted at a tertiary care teaching hospital located in South India, enrolled 332 adults at high risk for diabetes and subjected them to testing (FPG, 2 hPG, and HbA1c) twice at 2–3 weeks interval. Sensitivity of three tests for diagnosing diabetes and prediabetes was determined based on the final diagnosis of normoglycemia/prediabetes/diabetes made with six test results for each participant. Optimal HbA1c cutoffs for diabetes and prediabetes were determined based on the final diagnosis of glycemic status made with four test results of FPG and 2 hPG. Results: FPG, 2 hPG, and HbA1c, at American Diabetes Association recommended values, had sensitivity of 84.4%, 97%, and 93.8% respectively for diabetes diagnosis. HbA1c had lowest short-term variability (CVw = 1.6%). Receiver operating characteristic curve plotted with mean (of two values) HbA1c for each participant showed optimal HbA1c cutoffs of 6.5% for diabetes (area under curve [AUC] = 0.990, sensitivity = 95.8%, specificity = 96.2%, accuracy = 95.2%) and 5.9% for prediabetes (AUC = 0.893, sensitivity = 84.3%, specificity = 80%, accuracy = 75.6%) diagnosis respectively. HbA1c <5.6% had 100% negative predictive value to exclude prediabetes/diabetes. Conclusions: HbA1c ≥6.5% is a convenient and reliable alternative to plasma glucose tests to diagnose diabetes among high-risk South Indians. HbA1c ≥5.9% is optimal for prediabetes diagnosis and value <5.6% excludes prediabetes/diabetes.

Keywords: Asian Indians, diabetes, diagnosis, hemoglobin A1c, plasma glucose, prediabetes, repeat testing, variability

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Abbreviations used in the manuscript: ADA: American Diabetes Association, AUC: Area under curve, CVw: Within-person coefficient of variation, FPG: Fasting plasma glucose, 2 hPG: Two-hour plasma glucose post-75 g oral glucose load, HbA1c: Glycosylated haemoglobin, IFG: Impaired fasting glucose, IGT: Impaired glucose tolerance, NPV: Negative predictive value, PPV: Positive predictive value; PG: Plasma glucose, ROC: Receiver operating characteristic

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HbA1c offers some advantages over PG tests. It is more convenient to perform (can be done at any time of day and no need for overnight fasting and glucose challenge); and hence better compliance with testing, has lowest variability (unaffected by stress, acute illness and changes in diet and physical activity just before testing) and the values would not be affected by delayed analysis of blood samples after collection, unlike PG tests. However, HbA1c has some disadvantages such as higher cost, limited availability in the developing world, nonreliability in conditions such as hemolysis, significant anemia, hemoglobinopathy, pregnancy and renal disease, and lack of standardization of test across different laboratories. Although some Indian epidemiologic studies have compared performance of HbA1c with that of PG tests for diabetes and prediabetes diagnosis, the diagnosis was based on one-time testing and capillary PG has been used in some studies. ADA recommends venous PG and repeat testing (in view of variability of tests) within a short duration for the confirmation of diagnosis, unless there is unequivocal hyperglycemia. Variability of FPG, 2-h plasma glucose post-75 g oral glucose load (2 hPG) and HbA1c over a short duration has most probably not been studied in the Indian population so far. Moreover, it has been reported that strict application of guidelines for diabetes diagnosis and testing twice may give substantially different prevalence estimates compared with only a single measurement. With this background, the primary objective of present study was to compare sensitivity of HbA1c with that of FPG and 2 hPG for diabetes diagnosis among high-risk south Indians, as per the ADA criteria. The secondary objectives were to compare the sensitivity of three tests for prediabetes diagnosis, study the variability of tests over a short duration of 2–3 weeks and to determine optimal HbA1c cutoffs for diabetes and prediabetes diagnosis.

**Methods**

**Study design and participants**

This diagnostic accuracy study was carried out from December 2014 to July 2016, at a tertiary care teaching hospital located in South India and catering to both urban and rural population. Study participants were South Indians aged ≥18 years and had higher risk for diabetes compared to general population. Those risk factors were one or more of the following: age ≥45 years, abdominal obesity (waist circumference ≥90 cm in men and ≥80 cm in women), body mass index ≥23 kg/m², diabetes among biological parents or siblings, hypertension, physical inactivity, dyslipidemia (fasting serum triglycerides ≥150 mg/dl and/or high-density lipoprotein-cholesterol level <40 mg/dl in men and <50 mg/dl in women), known coronary artery or cerebrovascular disease and women with past gestational diabetes or delivery of a baby with birth weight ≥3.5 kg and polycystic ovarian syndrome. Exclusion criteria included previously diagnosed diabetes, overt hyperglycemic symptoms of diabetes, pregnant and lactating women, acute severe illness/hospitalization in preceding 2 weeks, hemoglobin concentration <10 g/dl, hemoglobinopathy, hemolytic anemia, major blood loss or history of blood transfusion in the past 6 months, serum creatinine ≥2 mg/dl, known connective tissue disease, thyroid disease, and concomitant drug therapy known to cause hyperglycemia such as corticosteroids, diuretics, and nicotinic acid. Study participation was sought from nonclinical hospital staff, relatives and friends of outpatients and those outpatients with hypertension, dyslipidemia, stable coronary artery disease, and cerebrovascular disease attending outpatient clinics of our hospital and convenience sampling was followed. The study was approved by the institute scientific and ethics committees. Written informed consent was obtained from those eligible and willing to participate in the study.

**Assessment of physical activity, fasting plasma glucose, 2-h plasma glucose post-75 g oral glucose load and HbA1c**

Physical activity was assessed according to the International Physical Activity Questionnaire scoring protocol and categorized into low, moderate, and high. Those with low-level physical activity were considered as sedentary. Participants were asked to come after overnight fast for ≥8 h. Fasting venous blood was drawn between 8 am and 10 am for measurement of FPG, lipids, and HbA1c. Participants subsequently ingested 75 g anhydrous glucose (83.3 g of Glucon-DTM, manufactured by Heinz India Ltd., Mumbai, India) dissolved in water and venous blood was collected again after 2 h for 2 hPG measurement. FPG, 2 hPG, and HbA1c tests were repeated at 2–3 weeks interval. Treatment of concomitant hypertension, dyslipidemia, and coronary/cerebrovascular disease did not change between visits. Participants were instructed not to change their diet and lifestyle during study and results of tests were disclosed to them only after the second visit. Blood was collected in sodium fluoride tubes for PG tests and analyzed within an hour of collection. PG was measured by glucose oxidase-peroxidase method, with Olympus AU400 Chemistry Analyzer (San Diego, California). HbA1c was measured by high-performance liquid chromatography, with Bio-Rad D10 analyzer (Bio-Rad Laboratories, California) and the coefficient of variation was 1.8%. HbA1c measurement was standardized, confirmed to National Glycosylated Hemoglobin Standardization Program and aligned to Diabetes Control and Complications Trial assay.
Statistical analysis
For assessing the difference between sensitivity of HbA1c and 2 hPG/FPG for diabetes diagnosis, sample size calculated was 331 (using the formula given below) with a power of 90%, an alpha error of 5% and an estimated sensitivity of 2 hPG and HbA1c of ~ 90% and 80% respectively.\(^7\)

\[
n = \frac{Z_\alpha \sqrt{2 \times P(1-P) + Z_\beta P(1-P) + P(1-P)}}{(P1-P2)^2}
\]

Statistical analyses were performed using SPSS Statistics-20 (IBM 1 Corp., Armonk, NY). For comparison of short-term variability of FPG, 2 hPG and HbA1c, we compared their values obtained at two visits for each participant. Differences were calculated as visit 1 minus visit 2, the within-person coefficients of variation were calculated as the square root of the within-subject variance divided by the mean squared and their confidence intervals were obtained using bootstrap methods.\(^10\)

Final diagnosis of glycemic status
FPG value \( \geq 126 \text{ mg/dl} (\geq 7 \text{ mmol/l}) \) and 2 hPG \( \geq 200 \text{ mg/dl} (\geq 11.1 \text{ mmol/l}) \) were used to diagnose diabetes. Impaired fasting glucose (IFG, FPG between 100 and 125 mg/dl, = 5.6–6.9 mmol/l) and/or impaired glucose tolerance (IGT, 2 hPG between 140 and 199 mg/dl, = 7.8–11 mmol/l) were used to diagnose prediabetes.

Each participant in the present study underwent 6 tests (FPG, 2 hPG, and HbA1c done twice). For calculating sensitivity of FPG, 2 hPG, and HbA1c for diabetes and prediabetes diagnosis in this study, we made the final diagnosis of diabetes, prediabetes or normoglycemia as follows. An individual was diagnosed to have diabetes if \( \geq 2 \) of 3 tests (FPG, 2 hPG, and HbA1c) done during any of two visits were suggestive of diabetes or any one of 3 tests was twice positive for diabetes during both visits, as recommended by ADA.\(^3\) For the purpose of this study, to have a more specific diagnosis of prediabetes (among those without diabetes), \( \geq 3 \) of 6 test results were required to suggest prediabetes. Those not fulfilling criteria for diabetes or prediabetes were considered normoglycemic.

Receiver operating characteristic (ROC) curves along with area under the curve (AUC) were used to characterize at HbA1c cutoffs to diagnose diabetes and prediabetes, with optimal sensitivity and specificity. For this purpose, we used 4 test results of FPG and 2 hPG to make the final diagnosis of diabetes/prediabetes/ normoglycemia as follows. Diabetes was diagnosed if \( \geq 2 \) of 4 tests were positive for diabetes. Among those without diabetes, prediabetes was diagnosed if \( \geq 2 \) of 4 tests were suggestive of prediabetes and the remaining were diagnosed to have normoglycemia.

Results
Initially, 433 high-risk individuals were screened for enrollment [Figure 1]. However, 332 finally completed the study and were included in the analysis. Table 1 shows the characteristics and risk factors for diabetes among study participants. Majority (253/332, = 76%) of participants were older than 45 years.

Sensitivity of fasting plasma glucose, 2-h plasma glucose post-75 g oral glucose load and HbA1c for diabetes and prediabetes diagnosis
Using 6 test results, we made the final diagnosis of diabetes in 96 (28.9%), prediabetes in 112 (33.7%) and normoglycemia in 124 (37.4%) participants, based on ADA criteria. Sensitivity of FPG [Table 2] to detect diabetes and prediabetes was 84.4% (81/96) and 40.2% (45/112) respectively. Similarly, the sensitivity of 2 hPG [Table 2] to detect diabetes and prediabetes was 97% (93/96) and 91.1% (102/112) and that of HbA1c [Table 3] was 93.8% (90/96) and 81.3% (91/112) respectively. There was good agreement between three tests for diabetes diagnosis [Figure 2]. Specificity of HbA1c value of 5.7%–6.4% for prediabetes diagnosis could not be calculated as there were some inconclusive diagnoses with HbA1c [Table 3]. However, around a third (38/124) with the final diagnosis of normoglycemia were falsely diagnosed to have prediabetes by HbA1c [Table 3].
Since HbA1c had very low variability (see below under short-term variability of tests) and there were some inconclusive diagnoses with two HbA1c results [Table 3], we calculated mean HbA1c for each participant. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of mean HbA1c of ≥6.5% to diagnose diabetes were 95.8%, 95.3%, 89.3%, and 98.3% respectively (accuracy = 95.5%).

**Short-term variability of fasting plasma glucose, 2-h plasma glucose post-75 g oral glucose load and HbA1c**

Table 4 gives the summary statistics for 3 tests at each visit, the differences between visits and the within-person coefficient of variation (CV_w) for each test. We assessed the degree of variability between two visits by calculating the difference between the mean values of these tests at each visit and by calculating the mean CV_w for each test. HbA1c had lower variability [CV_w = 1.6%; 95% confidence interval (CI), 1.5-1.8%] compared to FPG (CV_w = 7.5%; 95% CI, 6.9%-8.1%) and 2 hPG (CV_w = 6.1%; 95% CI, 5.7%-6.6%).

**Determination of optimal HbA1c cutoffs for diabetes and prediabetes diagnosis**

We plotted ROC curves of mean HbA1c (mean of two values for each participant) for both diabetes and prediabetes and deduced cutoffs with optimum sensitivity and specificity for their diagnosis. Based on 4 test results of FPG and 2 hPG, 95 (28.6%) participants were diagnosed to have diabetes and 108 (32.5%) prediabetes and ranges of mean

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**Table 2: Performance of fasting plasma glucose and 2-h plasma glucose post-75 g oral glucose load for diagnosis of diabetes and prediabetes (n=332)**

| Final diagnosis | Normoglycemia | Prediabetes | Diabetes | Total |
|-----------------|---------------|-------------|----------|-------|
| Normoglycemia (FPG <100 mg/dl or <5.6 mmol/L) | 123 (37) | 32 (9.6) | 0 | 155 (46.6) |
| Prediabetes (IFG) (FPG 100-125 mg/dl or 5.6-6.9 mmol/L) | 0 | 45 (13.6) | 3 (0.9) | 48 (14.5) |
| Diabetes (FPG ≥126 mg/dl or ≥7 mmol/L) | 0 | 0 | 81 (24.4) | 81 (24.4) |
| Inconclusive* | 1 (0.3) | 35 (10.5) | 12 (3.6) | 48 (14.4) |
| Total | 124 (37.4) | 112 (33.7) | 96 (28.9) | 332 (100) |

**Table 3: Performance of HbA1c for diagnosis of diabetes and prediabetes (n=332)**

| Final diagnosis | Normoglycemia | Prediabetes | Diabetes | Total |
|-----------------|---------------|-------------|----------|-------|
| Normoglycemia (<5.7%) | 55 (16.6) | 0 | 0 | 55 (16.6) |
| Prediabetes (5.7%-6.4%) | 38 (11.4) | 91 (27.4) | 4 (1.2) | 133 (40) |
| Diabetes (≥6.5%) | 0 | 0 | 90 (27.1) | 90 (27.1) |
| Inconclusive* | 31 (9.3) | 21 (6.3) | 2 (0.6) | 54 (16.2) |
| Total | 124 (37.4) | 112 (33.7) | 96 (28.9) | 332 (100) |

*Two values of HbA1c not in agreement for diagnosis of diabetes or prediabetes or normoglycemia. HbA1c: Glycosylated hemoglobin
HbA1c among them were 6.0–10.6% (42–92 mmol/mol) and 5.6%–6.8% (38–51 mmol/mol) respectively. HbA1c cutoff of >6.48% (=6.5%, 48 mmol/mol) was optimal for diabetes diagnosis (sensitivity = 95.8%, specificity = 96.2%, corresponding AUC of ROC curve = 0.990, PPV = 88.3%, NPV = 98.3%, diagnostic accuracy = 95.2%). HbA1c cutoff of >5.93% (=5.9%, 41 mmol/mol) was optimal for prediabetes diagnosis (sensitivity = 84.3%, specificity = 79.8%, AUC = 0.893, PPV = 58.3%, NPV = 92.3%, accuracy = 75.6%). HbA1c cut-off of <5.58% (=5.6%, 38 mmol/mol) excluded any degree of hyperglycemia (prediabetes/diabetes) with 100% NPV (specificity = 42.6%, PPV = 73.3%, accuracy = 77.7%).

**Discussion**

HbA1c, being a measure of average PG over prior 2–3 months, is a better marker of long-term hyperglycemia compared to FPG or 2 hPG tested twice and is better associated with chronic complications of diabetes,[1] especially microvascular ones. HbA1c serves both for diabetes diagnosis and monitoring of its control. However, HbA1c can be affected by age, race, ethnicity, erythrocyte environment and survival and genetic factors, which may give rise to hemoglobin glycosylation variability between individuals and groups.[8] In addition, concerns have been raised about the low sensitivity of HbA1c for diabetes diagnosis.[12-15]

It would be rather difficult to compare the results of present study with previous ones because the final diagnosis of glycemic status, unlike in most previous studies, was based on tests done twice. Hence, the diagnosis of diabetes and prediabetes in the present study was more definitive, and this assumes importance considering the need for lifelong treatment for diabetes, health-care costs involved and psychosocial impact on the diagnosed person. Moreover, the present study involved participants with multiple risk factors for diabetes [Table 1]. As per the diabetes risk assessment based on simple nonbiochemical parameters such as age, waist circumference, body mass index, family history of diabetes and physical activity validated in the previous studies involving Asian Indians,[16,17] most participants in the present study had a higher risk for diabetes compared to general population.

In the present study, 2 hPG had the highest sensitivity (97%) for diabetes diagnosis, followed closely by HbA1c (94%). FPG had the lowest sensitivity (84%) for diabetes diagnosis, similar to earlier studies.[18,19] HbA1c value ≥6.5% had high sensitivity for diabetes diagnosis in the present study, similar to earlier reports from South Indian population.[7,18] Higher inherent metabolic risk and higher HbA1c levels among South Asians[20] may account for high sensitivity of HbA1c in this study. However, much lower sensitivity of HbA1c of ≥6.5% for diabetes diagnosis has been reported in some Indian[6,8] and Malaysian[21] studies. In the present study, there was good agreement between diabetes diagnosed by HbA1c and the other two tests individually [Figure 2]. HbA1c diagnosed a different set of diabetes patients compared to 2 hPG and FPG in another South Indian study.[7] The frequency of diabetes and prediabetes in the present study was expected two to three times of their reported prevalence in earlier population-based studies from India,[6,7,9,22,23] because of higher metabolic risk among participants.[16,17] A Chinese study[24] involving high-risk subjects and another South Asian study[25] have reported similar high prevalence rates of diabetes and prediabetes.

2 hPG had the highest sensitivity (91%) for diagnosing prediabetes in the present study, followed by HbA1c of 5.7%–6.4% (81%). HbA1c sensitivity varying between 60% and 70%[9,18] and specificity of 77%[9] for detecting prediabetes have been reported in other Indian studies, at a cutoff of 5.7%. Unlike the present study, wherein HbA1c between 5.7% and 6.4% overdiagnosed prediabetes [Table 3], HbA1c cutoff of 5.7% underdiagnosed prediabetes in a previous study.[9] Since HbA1c alone overdiagnosed prediabetes in the present study [Table 3], the criterion used in the present study to make the final diagnosis of prediabetes (≥3 of 6 test results should be positive for prediabetes, after exclusion of diabetes) seems to be justifiable and led to a more specific diagnosis of prediabetes. 2 hPG has been found to be the most variable and HbA1c to be the least variable among diagnostic tests for diabetes in the previous studies.[5,10] HbA1c was the least variable of three diagnostic tests for diabetes over a short duration of 2–3 weeks in the present study, similar to earlier studies.[5,10] Thus, unlike PG tests, HbA1c may have the advantage of achieving diabetes diagnosis after single testing, provided the assay well-standardized and test is utilized judiciously considering its nonreliability in certain situations. Although HbA1c and FPG variability [Table 4] was comparable between the present and previous studies,[10,20] 2 hPG had much less variability in the present study compared

| Measurement | Visit 1 value | Visit 2 value | Mean difference between visits (visit 1- visit 2 value) | 95% limits of agreement* | CVw (95% CI) |
|-------------|--------------|--------------|--------------------------------------------------------|--------------------------|--------------|
| FPG (mg/dl) | 108.9 (31.7) | 109.8 (29.9) | -0.86 (13.6) | -27.5-25.8 | 7.5 (6.9-8.1) |
| 2 hPG (mg/dl) | 180.2 (64.6) | 180.69 (61.8) | -0.49 (17.98) | -35.7-34.8 | 6.1 (5.7-6.6) |
| HbA1c (%) | 6.5 (1.15) | 6.5 (1.12) | 0.01 (0.204) | -0.39-0.4 | 1.6 (1.5-1.8) |

*Mean±SD×1.96, †The CVw was calculated using the root mean square approach. SD: Standard deviation, CI: Confidence interval, FPG: Fasting plasma glucose, 2 hPG: 2-h plasma glucose post-75 g oral glucose load, HbA1c: Glycosylated hemoglobin, CVw: Within-person coefficient of variation.
Optimal HbA1c cutoff determined for diabetes diagnosis in the present study matched with ADA recommended cutoff of 6.5% and had high accuracy of ≈ 95%. Another south Indian study has reported optimal HbA1c cut-points of 6.4% and 6.1%, respectively, for diabetes diagnosed by FPG ≥126 mg/dl and 2 hPG ≥200 mg/dl criteria and 6.5% for diabetes diagnosed by both criteria, with diagnostic accuracy of >90%. The same authors suggested an optimal HbA1c value of ≥6.0% for diagnosing diabetes with high level of accuracy among Asian-Indians. HbA1c >6.3% was found to be optimal cutoff value for the diagnosis of type 2 diabetes in a study from Andhra Pradesh state in South India. A study from North India found an optimal HbA1c cut-point of 6.1% for diabetes screening and HbA1c of 6.5% had optimal specificity for diabetes diagnosis. Optimal HbA1c cutoff for prediabetes diagnosis in the present study was 5.9%, with a much lower accuracy compared to diabetes diagnosis. Optimal HbA1c cutoff for prediabetes (IFG or IGT) was 5.6% in another Indian study with accuracy <70%. These differences in the determined optimal HbA1c cutoffs for diabetes and prediabetes diagnosis in studies from different parts of India may be because of the ethnic diversity of India. A study of Han population from Northwest China showed optimal HbA1c thresholds of 6.4% and 6.1% for diabetes and prediabetes diagnosis respectively. One consistent finding in the present and previous studies is lower accuracy of HbA1c for prediabetes compared to diabetes diagnosis.

Strengths and limitations

This is probably the first study among Asian-Indians to study the variability of FPG, 2 hPG, and HbA1c over short duration. Testing twice, as per the ADA guidelines, enabled us to arrive at a more definitive diagnosis of diabetes and prediabetes. The hospital-based study and enrollment of high-risk subjects for diabetes may limit the generalizability of results from this study. However, determination of diabetes risk before testing and extrapolation of the present study results to subjects at moderate to high risk is suggested in this regard. Participants might have changed their lifestyle and diet during the study, which might have affected results of PG tests.

Conclusions

Among South Indians at high risk for diabetes, HbA1c of ≥ 6.5% is a convenient and reliable alternative to plasma glucose tests for diagnosing diabetes (accuracy ≥ 95%). Optimal HbA1c cutoff for prediabetes diagnosis was ≥5.9% (accuracy ≈ 75%) and a value of <5.6% may be used to confidently exclude prediabetes or diabetes. HbA1c, being the least variable of three diagnostic tests for diabetes, may achieve diagnosis after single testing. Further larger community-based studies from different parts of India, involving participants at different levels of risk for diabetes and employing tests twice, would be needed for ascertaining the role of HbA1c vis-à-vis plasma glucose tests.

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Conflicts of interest

There are no conflicts of interest.

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