Glycated Hemoglobin A_{1c} as Screening for Diabetes Mellitus in HIV-Infected Individuals

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Abstract

The American Diabetes Association now recommends hemoglobin A_{1c} (HbA_{1c}) screening for the diagnosis of diabetes. It has been reported that HbA_{1c} levels underestimate glycemic levels in HIV-infected persons. We examined the performance of HbA_{1c} as a screening test for diabetes in a group of HIV-infected people without diabetes. We conducted a retrospective cross-sectional cohort study among HIV-infected patients determining the sensitivity and specificity of HbA_{1c} as a screening test compared to fasting blood glucose (FBG). The effect of treatment regimen on the relationship between HbA_{1c} and FBG was assessed by multiple linear regressions. Twenty-two of the 395 patients included in the study were newly diagnosed with diabetes based on FBG $\geq$ 126 mg/dL. Using a cutoff of $HbA_{1c} \geq 6.5\%$, HbA_{1c} had a sensitivity of 40.9% and specificity of 97.5% for identification of incident diabetes. At an HbA_{1c} level of 5.8% the product of sensitivity and specificity was maximized, with values of 88.8% and 77.5% respectively. Higher mean cell volume (MCV) values ($p = 0.02$) and current use of a non-nucleoside reverse transcriptase inhibitors (NNRTIs; $p = 0.02$) significantly increased the slope, while PI use significantly decreased the slope ($p < 0.001$), of the linear regression of HbA_{1c} compared to FBG. Tenofovir use did not significantly alter the slope or y-intercept of the line. Among HIV-infected nondiabetic patients, HbA_{1c} is insensitive, although highly specific for diagnosing diabetes. Current antiretroviral (ART) use has significant and variable influence on the relationship between HbA_{1c} and FBG. The use of HbA_{1c} in conjunction with FBG may be the best modality to screen for diabetes.

Introduction

HIV-INFECTED INDIVIDUALS who are currently receiving antiretroviral therapy (ART) appear to be at increased risk of developing diabetes mellitus.[1–4] As a result, international guidelines recommend yearly screening of all HIV-infected individuals for diabetes.[5,6]

In 2009 the American Diabetes Association (ADA) introduced glycated hemoglobin A_{1c} (HbA_{1c}) as an alternative to fasting blood glucose (FBG) and 2-h oral glucose tolerance tests (OGTT) in screening for diabetes. They defined diabetes as having an $HbA_{1c} \geq 6.5\%$.[7,8] As with FBG and OGTT, this cut-off was based on increasing risk of retinopathy.[6] The use of HbA_{1c} for screening (1) provides the clinician with the ability to estimate the average level of glycemia and (2) provides a measure that is accurate in a nonfasting state. ADA guidelines suggest HbA_{1c} is an acceptable screening test unless there are abnormalities in erythrocyte structure (e.g.,

hemoglobinopathies) or turnover (e.g., pregnancy, significant bleeding, hemolysis, or iron deficiency anemia).

Reports of HbA_{1c} levels in HIV-infected people with diabetes consistently demonstrated that HbA_{1c} underestimated the level of glycemia.[9–12] The reason for the discordant HbA_{1c}-FBG relationship observed in HIV-infected patients is still unclear. The existing data on HbA_{1c} in HIV-infected patients are in known diabetics for monitoring of glycemic control, prior to the widespread use of tenofovir. In this study we compared HbA_{1c} and FBG as screening tests for the diagnosis of diabetes mellitus in the HIV-infected population.

Methods

Study design and participants

In 2009, providers at the Bellevue Virology Clinic began screening for diabetes using HbA_{1c} as part of a quality

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improvement initiative. Charts of all HIV-infected patients 18 years of age or older who had one or more HbA_{1c} values recorded in the hospital's electronic records between October 2009 and March 2010 were reviewed for inclusion in this retrospective cross-sectional cohort study. Patients were excluded from the study if they had underlying conditions or were on medications that alter either red blood cell life span or blood glucose levels. Conditions for exclusion included known diabetes (defined by current use of antihyperglycemic therapy), hemoglobinopathies, G6PD-deficiency, pregnancies, hospitalization or receipt of a transfusion within the prior 3 months, treatment with systemic corticosteroids, or absence of a FBG value in the 3 months prior to HbA_{1c} testing. Approval for this study was received from the Institutional Review Board of the NYU School of Medicine, from Bellevue Hospital Center, and from the central office of the NY City Health and Hospital Corporation.

Measures

Demographic characteristics, current medications (including ART), most recent HIV viral load, CD4 count, and red blood cell indices were extracted from each patient's chart. The FBG used for comparison with the HbA_{1c} was the average of FBG values obtained during the 3 months prior to HbA_{1c} testing. At the study site, patients are asked if they are fasting prior to blood testing, and those in a nonfasting state are asked to return on a subsequent day for testing when they are fasting. FBG was measured in a Siemens ADVIA 2400 analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) using the Hexokinase method, and HbA_{1c} values were determined by the high-performance liquid chromatography (HPLC) assay of Primus diagnostics (Trinity Biotech, Bray, Ireland). Both tests were performed at Bellevue Hospital Center's Clinical Laboratory.

We defined FBG as elevated if it was $\geq 126\,mg/dL$ and HbA_{1c} as elevated if it was $\geq 6.5\%$, in accordance with laboratory cutoffs and recommendations of the ADA.[7,8,13] We assessed the sensitivity and specificity of HbA_{1c} as a screening test for diabetes using FBG as a gold standard. Additionally, patient variables (erythrocyte characteristics, race, and current ART) and their effect on both the test characteristics of HbA_{1c} (sensitivity and specificity), and the relationship between HbA_{1c} and FBG values were examined. The subset sensitivities and specificities were calculated using the accepted cut-point for diagnosing diabetes ($HbA_{1c} \geq 6.5\%$).

Statistical analyses

Data were analyzed using both SPSS version 19 (SPS Inc., Chicago, IL) and the R statistical environment.[14] A receiver operating characteristic (ROC) curve was computed to evaluate the performance of HbA_{1c} as a screening test for the full cohort using various cut-points. Pearson's uncorrected χ^2 test was used to assess the significance of the differences among sensitivities and specificities of HbA_{1c} level, for various subsets of the cohort.

The effect of erythrocyte characteristics, race, and current ART on the relationship between HbA_{1c} and FBG were assessed by multiple linear regression. Regression models included FBG as the dependent variable and HbA_{1c} as one independent variable, together with variables representing the characteristic under study and its interaction with HbA_{1c}.

A characteristic was said to affect the relationship between FBG and HbA_{1c} if inclusion of the latter two terms in the model significantly reduced the regression's residual variance as judged by an appropriate F ratio. Additional F ratios were examined after a positive result to assess the significance of changes in slope or y-intercept. Model fit was assessed by graphic examination of residuals and influence statistics.

Results

Four hundred ninety HIV-infected patients had HbA_{1c} results available during the study period, and 395 were included in the analysis. Of the 95 patients excluded, 39 were known to have diabetes and receiving antihyperglycemics, 22 had a hemoglobinopathy or G6PD deficiency, 3 were pregnant, 2 were receiving systemic corticosteroids, 40 had a recent hospitalization, and 19 had no FBG value in the 3 months preceding HbA_{1c} testing. Baseline characteristics of the study population are displayed in Tables 1 and 2. The mean age of patients was 46.2 years, 77.5% were male, and the majority belonged to racial or ethnic minorities (46.8% black and 34.9% Hispanic).

Of the 395 patients included in the study, 13 (3.3%) had an elevated FBG and normal HbA_{1c}, 9 (2.3%) had an elevated HbA_{1c} and a normal FBG, and 9 (2.3%) were concordant for elevations in both tests. Relative to the FBG gold standard, the sensitivity of HbA_{1c} as a screening test for diabetes was 40.9% and the specificity was 97.5%. Based on the ROC curve the optimal cut-point, defined as the value that maximizes the product of sensitivity and specificity, was a HbA_{1c} level of 5.8%, which was 81.8% sensitive and 77.5% specific (Fig. 1). Subgroup analysis by ART [categorized as either no ART or by receipt of regimen containing an nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), or protease inhibitor (PI)] showed no

TABLE 1. CHARACTERISTICS OF STUDY PARTICIPANTS AT INDEX VISIT

n[a]	395
Demographics	
Age (mean ± SD)	46.2 ± 10.9
Gender (% male)	77.5
Race (%)	
Black	46.8
Hispanic	34.9
White	7.3
Asian/Pacific Islander	7.8
Other	2.3
Unknown	0.8
HIV disease characteristics	
CD4 count (mean ± SD)	445.0 ± 250.4
Viral load (% undetectable)	66.8
Laboratory measures	
HbA_{1c}	
Meadian (IQR)	5.5 (5.3–5.8)
Mean ± SD	5.6 ± 0.8
FBG	
Values per patient[b] (mean ± SD)	2.01 ± 0.88
Median (IQR)	91 (84–99)
Mean ± SD	96.0 ± 28.5

[a]Number of participants.
[b]Average number of FBG values per patient included in analysis.
SD, standard deviation; Hba_{1c}, glycated hemoglobin A_{1c}; IQR, interquartile range; FBG, fasting blood glucose.

	n^a
No antiretrovirals	63
Nucleoside/nucleotide reverse transcriptase inhibitor	319
Abacavir	49
Didanosine	9
Emtricitabine	235
Lamivudine	72
Stavudine	4
Tenofovir	269
Zidovudine	41
Non-nucleoside reverse transcriptase inhibitor	136
Efavirenz	116
Etravirine	12
Nevirapine	8
Protease Inhibitor	188
Ritonavir[b]	181
Atazanavir	85
Darunavir	29
Fosamprenavir	17
Indinavir	2
Lopinavir	51
Nelfinavir	4
Saquinavir	2
Tipranavir	0
Integrase inhibitor/raltegravir	40
Entry inhibitor	
Enfuvirtide	1
Maraviroc	6

[a]Number of participants receiving each treatment.
[b]Of patients on protease inhibitors only 7 were not receiving ritonavir boosting; 4 patients taking nelfinavir, 2 taking atazanavir, and 1 taking fosamprenavir.
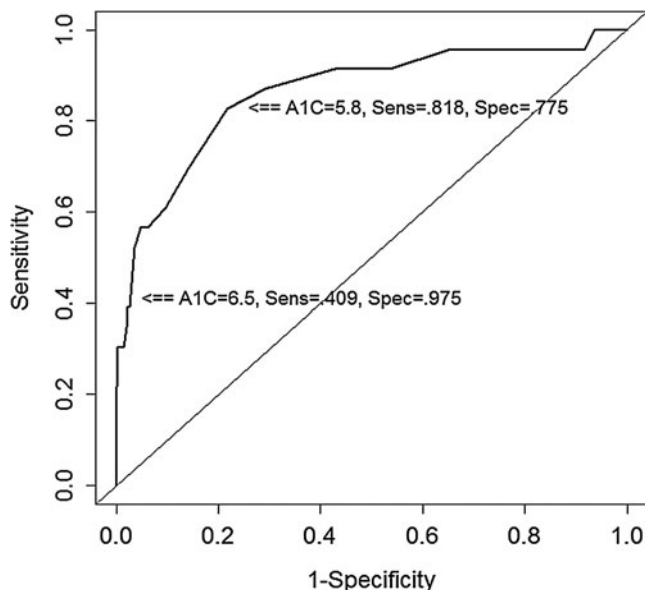

FIG. 1. Receiver operating characteristic (ROC) curve for HbA$_{1c}$ levels as a diagnostic indicator for diabetes [defined as a fasting blood glucose (FBG) $\geq$ 126 mg/dL]. A$_{1c}$, glycated hemoglobin A$_{1c}$ (HbA$_{1c}$); sens, sensitivity; spec, specificity.

statistically significant effect on sensitivity or specificity (data not shown).

Of the 13 patients with an elevated FBG and normal HbA$_{1c}$, 4 were found to have been previously diagnosed with diabetes, 3 had normal FBG determinations on a subsequent visit, and 6 were new cases of diabetes and were started on either medical or lifestyle management. None of the 4 previously diagnosed diabetics were on antihyperglycemic therapy, 1 had a prior history of indinavir-induced diabetes, 1 had previous steroid-induced diabetes, and 2 were known diabetics off treatment. Of the 9 patients with an elevated HbA$_{1c}$ and a normal FBG, only 1 was found to have a prior history of elevated FBG levels, and 4 of the 9 had a subsequent HbA$_{1c}$ value < 6.5%.

Multiple linear regression confirmed that mean cell volume (MCV) affected the relationship between FBG and HbA$_{1c}$ (F test for interaction between MCV and A$_{1c}$ = 5.4, $p = 0.02$) by increasing the slope of the regression curve. Hemoglobin level had no significant effect on this relationship, nor did racial identification as black versus nonblack (data not shown).

Current use of a PI was associated with a significantly decreased slope for the regression of FBG on HbA$_{1c}$ (F = 17.02, $p < 0.001$), suggesting that for patients receiving a PI, an elevated HbA$_{1c}$ may overestimate the actual level of glycemia (Fig. 2). Current use of an NNRTI was associated with both a significantly increased slope (F = 5.16, $p = 0.02$) and a significantly decreased y-intercept (F = 6.69, $p = 0.01$) for the regression of FBG on A$_{1c}$, suggesting that elevated HbA$_{1c}$ values in these patients may underestimate the level of glycemia. Use of an NRTI reduced the y-intercept (F = 5.44, $p = 0.02$) but had no significant effect on the slope (F = 0.64, $p = 0.42$). Tenofovir use did not significantly impact the slope (F = 1.23, $p = 0.27$) nor the y-intercept (F = 0.004, $p = 0.95$) of the regression curve. We did not analyze the effects of other individual antiretrovirals because of the smaller numbers of patients receiving each agent.

Discussion

Routine follow-up of HIV patients involves screening and monitoring for metabolic complications of ART including dyslipidemia and hyperglycemia. New guidelines from the American Diabetes Association recommend HbA$_{1c}$ as a screening test for diabetes. However previous studies have suggested HbA$_{1c}$ underestimates the level of glycemia in patients with HIV infection and diabetes.

In our cross-sectional cohort study we found that an HbA$_{1c}$ $\geq$ 6.5% is insensitive, but highly specific in diagnosing diabetes among HIV-infected patients. The ideal cut-point on the ROC curve to yield the highest combination of sensitivity and specificity is 5.8%. This cut-point value is identical to that found in the general population through the NHANES cohort,[15] and the sensitivity and specificity of the test was not significantly affected by antiretroviral regimen.

We identified 22 patients with discordant HbA$_{1c}$ and FBG values (13 false-negative, and 9 false-positive HbA$_{1c}$ values). Newly published recommendations from the ADA acknowledge that such conflicting laboratory tests are not an uncommon finding.[8] The position statement cites unpublished NHANES data that indicates using a HbA$_{1c}$ cut-point of $\geq$ 6.5% will diagnose one third fewer cases of diabetes compared to a FBG $\geq$ 126 mg/dL. As of 2011 the ADA altered
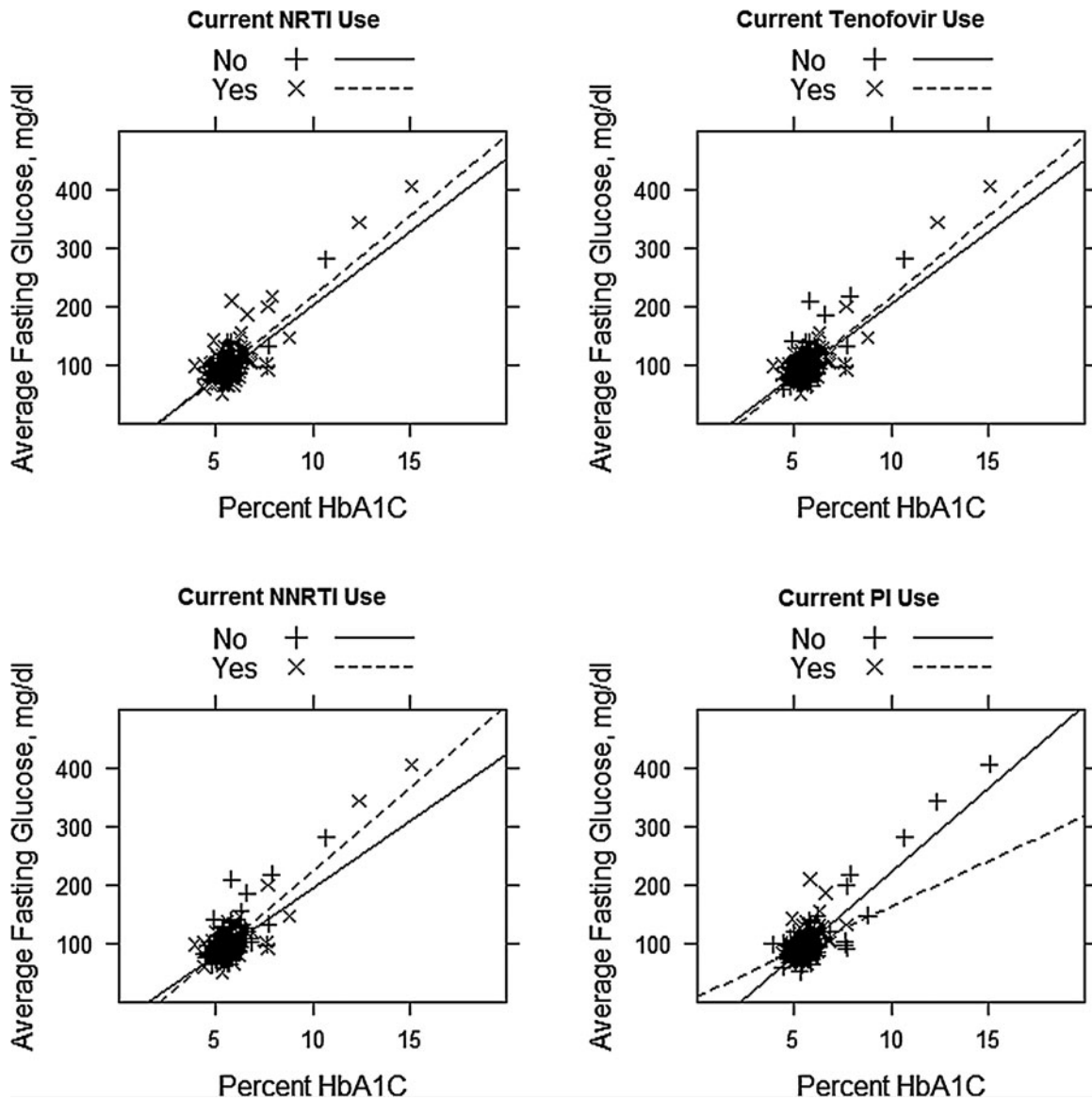
FIG. 2. Scatter plot and regression curves of fasting blood glucose and hemoglobin A_{1c} (HbA$_{1c}$) adjusted for current antiretroviral use. NRTI, nucleoside reverse transcriptase inhibitor; NNTRI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

its recommendations to say that in cases of HbA$_{1c}$ and FBG discordance, the abnormal laboratory test should be repeated, and the diagnosis of diabetes should only be made if repeat testing is again above the diagnostic cut-point.[8] Had we used these new recommendations to define diabetes in our study, we would have reduced the number of false-positive HbA$_{1c}$ values from 9 to 4, and decreased the number of false-negative tests from 13 to 12; resulting in an improved sensitivity and specificity of 42.9% and 98.7%, respectively.

Similar to previous studies,[10–12] higher MCV resulted in lower HbA$_{1c}$ values than would be expected for the FBG level. Although thymidine analogues are well-documented to increase MCV[16,17] we had only a small fraction of our patients on these agents (stavudine or zidovudine). Unlike previously published studies in the general population,[18] our analysis did not show any significant effect of black race on the relationship between HbA$_{1c}$ and FBG. These results held even when including patients with either hemoglobinopathies or G6PD-deficiency (data not shown).

Our findings support the idea that HbA$_{1c}$ values are affected by current antiretroviral regimen. These findings are different from previous studies on diabetic patients that showed no statistically significant effect of PIs or NNRTIs on HbA$_{1c}$ levels.[10,12] Our results suggest that HbA$_{1c}$ values in HIV-infected patients currently receiving PIs may overestimate, and those currently receiving NNRTIs may underestimate, the level of glycemia. In contrast tenofovir use did not appear to impact the relationship between HbA$_{1c}$ and FBG. The number of patients on abacavir was insufficient to determine an affect on the relationship between HbA$_{1c}$ and FBG as seen in prior studies.[11]

Limitations of our study include its small sample size, lack of long-term clinical outcomes, and use of fasting blood glucose (as opposed to OGTT) as the gold standard to which

HbA$_{1c}$ was compared. Additionally FBG levels in our study were assumed to be fasting. Although the clinic procedure used at the study site attempts to ensure fasting blood testing, the retrospective use of routine laboratory testing may have allowed for the inclusion of nonfasting samples.

In conclusion, there is growing evidence about the variable accuracy of HbA$_{1c}$ as a screening test for diabetes. We observed that HbA$_{1c}$ is an insensitive, but highly specific screening test for diabetes in HIV-infected patients. The relationship of HbA$_{1c}$ to FBG is affected by specific antiretrovirals, but not enough to significantly alter the sensitivity or specificity of the test. More work is needed to determine the clinical significance and mechanism behind specific antiretroviral drug's impact on HbA$_{1c}$. Despite the possible limits on its interpretation, the ability to use HbA$_{1c}$ to screen HIV-infected patients in a nonfasting state and estimate long-term glycemic levels makes it a practical screening modality, and we advocate the use of HbA$_{1c}$ and FBG in conjunction to guide the clinician in screening for diabetes.

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Author Disclosure Statement

No competing financial interests exist.

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