

The role of HbA1c as a screening and diagnostic test for diabetes mellitus in Ankara

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Aim: This study investigated the value of HbA1c as a screening and diagnostic test for diabetes mellitus (DM) in high-risk Turkish individuals.

Materials and methods: A total of 295 participants were successfully screened. Patients were divided into 4 groups based on their oral glucose tolerance test results, according to criteria put forth by the American Diabetes Association; 120 (40.7%) had normoglycemia, 44 (14.9%) had DM, 62 (21%) had impaired fasting glucose, and 69 (23.4%) had impaired glucose tolerance.

Results: With a cut-off value for the diagnosis of DM of 6.1%, HbA1c had a sensitivity of 81.8% and a specificity of 80%, with positive and negative predictive values of 80.2% and 81.05%, respectively. A sensitivity of 56.8% and a specificity of 89.2% were calculated for a cut-off value of 6.5%. Both fasting plasma glucose and 2-h plasma glucose levels were found to correlate moderately with HbA1c levels ($r = 0.47$, $P = 0.001$ and $r = 0.52$, $P = 0.000$, respectively).

Conclusion: The results of our observations suggest that HbA1c could be used to make a diagnosis of DM in the Turkish population. However, further studies are needed to determine the most accurate cut-off value. Standardization of HbA1c assays used worldwide is also of great importance.

Key words: HbA1c, diabetes mellitus, diagnosis, oral glucose tolerance test

Introduction

Diabetes mellitus (DM) is one of the most commonly encountered chronic disorders. By 2030, the worldwide prevalence of adult DM is expected to rise to 7.7%, which roughly translates to 439,000,000 affected individuals (1). Based on current recommendations, a diagnosis of DM requires the presence of a fasting plasma glucose concentration of ≥ 126 mg/dL, or a 2-h plasma glucose level of ≥ 200 mg/dL on an oral glucose tolerance test (OGTT). On the other hand, international committee members selected by the American Diabetes Association (ADA) and the Alliance for European Diabetes Research (EURADIA) recently suggested that glycosylated

hemoglobin (HbA1c) could be used as an alternative for making a diagnosis. (2). The committee concluded that an HbA1c level of $\geq 6.5\%$ was diagnostic for DM, without requiring a determination of blood/plasma glucose levels. However, the use of standard glucose measurements is still recommended for individuals when HbA1c assays are deemed unreliable (3)

HbA1c is formed as a result of the addition of a stable glucose molecule to the N-terminal group of an HbA₀ molecule via a nonenzymatic glycation process (4), and is considered a reliable indicator of the glycemic status of the previous 3 months (5).

Despite the cloud of controversy regarding the limitations of HbA1c for making a diagnosis of DM,

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many experts believe that HbA1c may be superior to the OGTT in daily clinical practice. The Turkish Endocrine Society does not consider HbA1c a reliable diagnostic test for DM, citing insufficient standardization of available HbA1c assays as well as the inconclusive results concerning the optimal cut-off value(6).

The aim of this study was to investigate the value of HbA1c as a screening and diagnostic test for DM in high-risk Turkish individuals.

Materials and methods

Patient selection and initial evaluation

This study was undertaken in the Department of Endocrinology at Dışkapı Yıldırım Beyazıt Training and Research Hospital with the approval of the local ethics committee. Patients with known risk factors for developing type 2 diabetes mellitus (T2DM), such as a family history of DM or impaired fasting glucose, who presented to the outpatient clinic between September 2010 and April 2011 were approached for inclusion in this study, and consenting patients were enrolled. A detailed history was obtained for each patient, followed by a thorough physical examination including anthropometric measurements and determination of arterial blood pressure. Height, weight, waist circumference, and systolic and diastolic blood pressure measurements were recorded for each patient, and body mass indices were calculated using the formula $BMI = (\text{weight in kg} / \text{height in m}^2)$.

Blood sampling and laboratory assays

For each patient, blood samples were obtained at 0800 hours following a 12-h fast, from the antecubital vein in a sitting position, for the determination of HbA1c as well as baseline/fasting blood glucose level. All patients were then subjected to a 75-g OGTT (on the same day), and second blood samples were obtained 2 h after glucose loading.

HbA1c measurements were made using an NGSP-approved latex agglutination inhibition method on an Advia 2400 (Siemens Healthcare Diagnostics, Germany) analyzer. Blood samples were first incubated for at least 5 min with a hemoglobin-denaturing reagent to allow for lyses of erythrocytes. Proteases and hemoglobin chains within the resultant mixture were then hydrolyzed followed by

determination of total hemoglobin levels. HbA1c levels were determined by agglutination with anti-HbA1c antibodies. The intraassay coefficients of variation for normal and abnormal patients were 1.2% and 0.8%, respectively, with respective total coefficients of variation of 2.0% and 1.8%. Glucose measurements were made on the same day as HbA1c measurements, using the glucose oxidation method on an Advia 2400 analyzer. Subjects were categorized into 4 groups based on their OGTT results, according to criteria put forth by the ADA: normoglycemic, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and DM.

Statistical analyses were performed using SPSS 17. Values for HbA1c and blood glucose levels are provided as mean \pm standard deviation. The sensitivity, specificity, negative predictive, and positive predictive values for both tests were calculated by plotting a receiver operating characteristic (ROC) curve. Correlation analyses between fasting plasma glucose, 2-h plasma glucose, and HbA1c levels were performed using Spearman's correlation test. A P-value of less than 0.05 was considered indicative of statistical significance.

Results

A total of 295 consenting participants (79 male and 216 female) were included in the final analysis. Based on OGTT results and according to criteria put forth by the ADA, 120 individuals were categorized as normoglycemic, 44 had DM, 63 had IFG, and 69 had IGT. The demographic characteristics and laboratory findings of the study population have been summarized in the Table.

The OGTT was considered the gold-standard test for the diagnosis of DM. The area under the ROC curve for the diagnosis of DM was 0.85 ($P < 0.001$) (Figure 1). With a cut-off value of 6.1%, HbA1c had a maximal sensitivity and specificity of 81.8% and 80%, respectively, with a positive predictive value (PPV) of 80.2% and a negative predictive value (NPV) of 81.05%. With a cut-off value of 6.5%, the sensitivity of HbA1c was 56.8% with a specificity of 89.2%.

The area under the curve (AUC) for impaired glucose tolerance was 0.67 ($P < 0.001$). HbA1c had a sensitivity of 63.8%, specificity of 60%, PPV of 61.4%

Table. Demographic characteristics and laboratory findings of study population.

Parameter	Total N = 295	NG N = 120	DM N = 44	IFG N = 62	IGT N = 69
Age, years	48.98 ± 12.22	45.97 ± 12.22	52 ± 12.39	50.32 ± 10.70	51.11 ± 12.50
Fasting plasma glucose	101.4 ± 19.60	89.16 ± 11.56	139.88 ± 18.71	108.09 ± 6.89	102.31 ± 14.68
2-h plasma glucose	140.81 ± 61.33	100 ± 19.73	255.5 ± 56.32	110.53 ± 20.99	165.60 ± 17.18
HbA1c, %	6.0 ± 0.80	5.75 ± 0.73	6.80 ± 0.93	5.83 ± 0.57	6.08 ± 0.68
BMI, kg/m ²	30.47 ± 5.55	28.93 ± 6.00	32.39 ± 4.67	31.38 ± 4.96	30.93 ± 4.90
SBP, mm/Hg	124.24 ± 16.42	121.58 ± 14.68	128.81 ± 19.65	125.32 ± 16.61	125.94 ± 14.21
DBP mm/Hg	77.39 ± 11.13	76.25 ± 10.94	77.88 ± 13.47	78.46 ± 10.02	79.24 ± 10.48
WC, cm	96.48 ± 15.66	91.67 ± 13.87	102.20 ± 15.08	97.19 ± 15.14	102.75 ± 16.23

Values provided as mean ± standard deviation; BMI, body mass index; HbA1c, glycosylated hemoglobin; NG, normoglycemic group; DM, overt diabetes mellitus group; IFG, impaired fasting glucose group; IGT, impaired glucose tolerance group; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference.

and NPV of 62.37% with a cut-off value of 5.7%. A positive correlation was observed between HbA1c, fasting plasma glucose ($r = 0.47$, $P = 0.001$), and 2-h plasma glucose ($r = 0.52$, $P = 0.000$) glucose levels in patients with DM (Figures 2 and 3).

Discussion

Recently, the ADA has recommended that HbA1c be used for the diagnosis of DM with a cut-off value of $\geq 6.5\%$, taking into consideration the strong association between this cut-off value and the prevalence of retinopathy (2). A similar association between HbA1c and risk of developing both diabetes and cardiovascular disease has also been reported in adults (7).

The accuracy and precision of HbA1c assays has improved greatly in recent years with the advent of technological advancements and widespread availability of international standardization (8). Determination of HbA1c has now become more advantageous than plasma glucose measurements, since HbA1c is biologically more stable and remains largely unaffected in the short term by nutritional status, stress, or other disorders (9)

Several problems are associated with blood glucose measurements, such as interindividual biological variations and preanalytic variables like sampling method (e.g., at room temperature, glucose levels decrease by 3–8 mg/dL per hour) and fasting

status prior to blood sampling. Pretest exercise and calorie restriction are other factors that may affect interpretation of results (10). HbA1c does not require fasting and has an analytical variation of less than 2%, which overcomes most of the difficulties mentioned above. The main disadvantages of this test are the high cost and the need for determination by a method certified by the NGSP, such as high-performance liquid chromatography (HPLC). HbA1c shows very little daily variation and is widely believed to be a more suitable reflection of chronic glycemic status (11).

Kumar et al. (11) reported on a sensitivity of 65% and a specificity of 88%, with positive and negative predictive values of 75.2% and 96.5%, respectively, with a cut-off value of 6.5%. Peter et al. (12) reported that an HbA1c value of 6.5% could distinguish diabetic patients from nondiabetic patients with a specificity of 98.7%. However, in the same study, the investigators observed a very low sensitivity of 46.8%, which translates into a missed diagnosis in more than half of the patients who actually have DM. In our study population, an HbA1c value of 6.5% had a sensitivity of 56.8% for making a diagnosis of DM, a finding consistent with results of previous reports.

The low sensitivity of HbA1c as a diagnostic test for DM could be attributed to several factors, such as the presence of iron deficiency anemia or another disorder affecting hemoglobin turnover, as well as several hereditary and environmental factors (13).

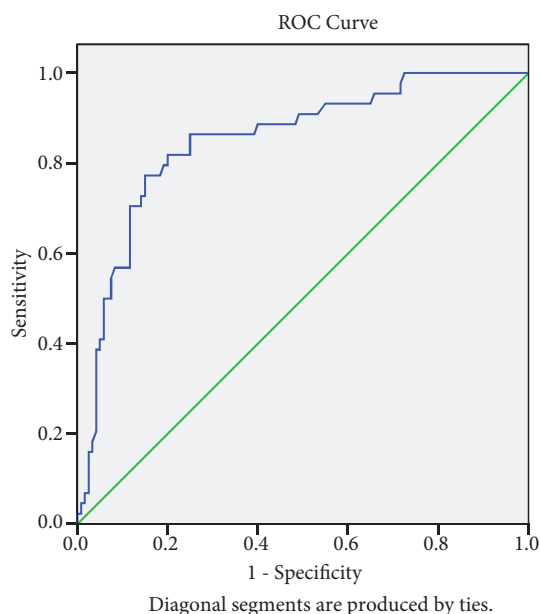


Figure 1. Receiver operating characteristic curve for HbA1c in patients with diabetes mellitus.

Nearly 50% of variations in HbA1c levels may be explained by changes in blood sugar profile (14). While HbA1c is a measure of chronic glycemic control, determination of blood glucose levels only represents glucose concentration at the time of sampling and may not correctly reflect a patient's glycemic status over the previous months. Furthermore, results of the OGTT may be influenced by the presence of an active infection, physical activity, and diet (12). All patients were informed regarding the possible effects of such confounding factors, and blood sampling was scheduled taking these factors into consideration.

Several studies have investigated the value of HbA1c for the diagnosis of DM with a cut-off level of 6.1%. In such a study by Tavintharan et al. (15), a sensitivity of 81% was reported with a specificity of 84%. Similarly, Ko et al. (16) reported on a sensitivity and specificity of 77.5% and 78.8%, respectively. Comparable results were observed in our study with a cut-off value of 6.1% (sensitivity, 81.8%; specificity, 80%; PPV, 80.2%; NPV, 81.05%).

Numerous studies have attempted to investigate the role of HbA1c as a diagnostic test for DM, by comparing it with the OGTT as the gold-standard (17–19). Colagiuri et al. (17) evaluated the screening strategies that included measurement of HbA1c

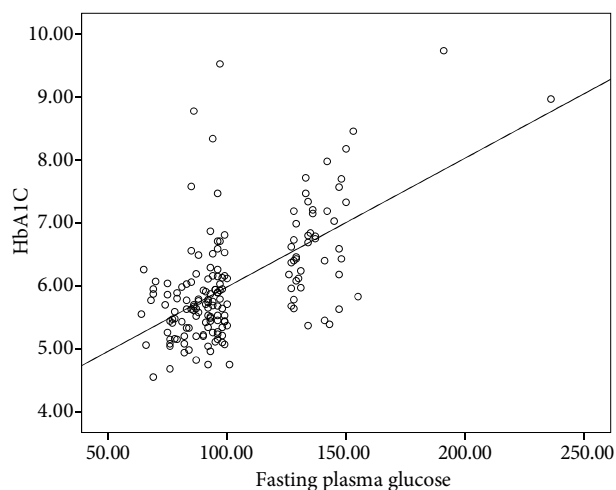


Figure 2. Correlation curve for fasting plasma glucose and HbA1c ($r = 0.47$, $P = 0.001$).

in a population ($N = 10,447$) without previously diagnosed diabetes. In their study, a cut-off value of 5.3% for HbA1c had a sensitivity of 78.7% with a specificity of 82.8% (17). Van't Riet et al. (20) also reported on a sensitivity of 72% with a specificity of 91% in a study of 2708 patients where a cut-off value of 5.8% was used. The significantly lower number of participants in our study compared to the Colagiuri and van't Riet studies may explain the discrepancies in our findings, although our results are comparable to those reported in a study by Kumar et al. (11) on individuals whose DM status was unknown, where a cut-off value of 6.1% was used.

Santos-Rey et al. (21) investigated the role of HbA1c with a cut-off value of 5.4% for the diagnosis of IGT. They reported a sensitivity of 85%, a specificity of 73%, and a NPV of 97%. A similar analysis in our study with a cut-off value of 5.7% produced a sensitivity of 60% with a specificity of 63.8%, a PPV of 61.4%, and a NPV of 62.37%. Results of studies on the potential role of HbA1c for detecting individuals at risk of developing IGT remain inconclusive, and further studies are needed to fully elucidate its value as a diagnostic test.

Van't Riet et al. (20) reported on a significant, albeit moderate, correlation between HbA1c and fasting plasma glucose ($r = 0.57$) and 2-h plasma glucose ($r = 0.35$) levels. It was suggested that

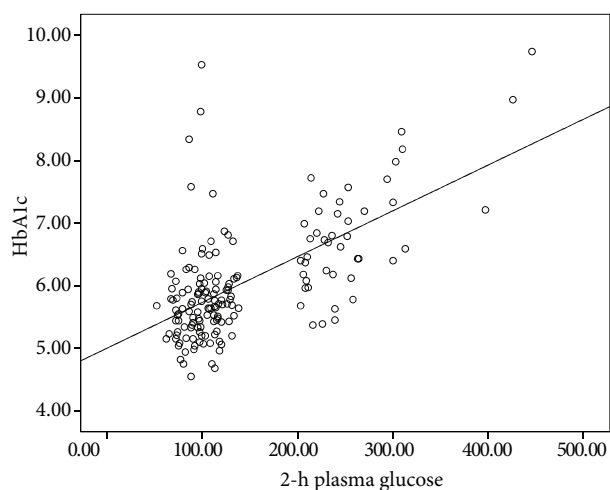


Figure 3. Correlation curve for 2-h plasma glucose and HbA1c ($r = 0.52$, $P = 0.000$).

HbA1c and glucose go through different processes, particularly during the period between impaired glucose tolerance and the development of overt DM. The extent of glycosylation is known to show individual variability. Potential mechanisms include genetic characteristics (22), age (23), variations in surrounding conditions of erythrocytes (24), heterogeneity in the life cycle of erythrocytes, and ethnic variations. We also managed to demonstrate a statistically significant positive correlation between HbA1c and fasting plasma glucose ($r = 0.47$) and 2-h plasma glucose ($r = 0.52$) levels.

Riet et al. (20) reported that weak positive correlations between HbA1c and fasting plasma glucose levels ($r = 0.46$), as well as 2-hour plasma glucose ($r = 0.33$) levels, were observed in individuals from the general population. The correlation between HbA1c and fasting plasma glucose and 2-h plasma glucose levels was weaker in patients with IFG or IGT compared to those with overt DM ($r = 0.38$ and $r = 0.43$, respectively). Although a wide range of values have been reported for both glucose and HbA1c in the general population, the correlation between them is stronger in patients with DM, a finding that has also been reiterated by van't Riet et al. (20).

OGTT and HbA1c results may sometimes show discordance, which is not explainable by analytical variations alone. Such discrepancies may be attributed to biological variations. Evaluation of HbA1c levels still suffers from the lack of an international standard,

as well as the neglecting of several cofactors such as age, sex, and ethnic characteristics (25).

In a study from Turkey, Köşüş et al. (26) recommended the glucose challenge test (GCT) as an international screening method and suggested that the GCT was also suitable for Turkish women. The place of residence as well as race needed to be taken into consideration to establish the best cut-off level for the GCT, since ethnic and environmental factors might contribute to the occurrence of gestational DM.

Different ethnic groups were found to have different sensitivity and specificity values for HbA1c as a diagnostic test for DM, which may be related to genetic differences in the concentration of hemoglobin, the rates of glycation, and the lifespan or amount of red blood cells (27). Recently, racial and ethnic variations in HbA1c have been reported to impact the potential utility of HbA1c as a diagnostic test for diabetes (28). Ethnic variations in HbA1c could not be evaluated in our study since all participants were living in the Ankara area (29). While HbA1c is a reliable indicator of chronic glycemic status, 2-h glucose measurements obtained from an OGTT are considered to reflect daily insulin secretion. HbA1c is expected to overtake the OGTT as the test of choice for DM, which, when it does occur, would be considered a milestone in the management of DM (12).

Many studies have demonstrated that HbA1c correlates better with microvascular complications in patients with DM (29). Zengin et al. (30) suggested that higher HbA1c levels as a marker of poor glycemic control were associated with thicker corneas in T2DM. On the other hand, insulin resistance is a better predictor for the development of macrovascular complications (31). Insulin resistance is even considered to be the most important risk factor for the development of coronary artery disease (32). In another study with conflicting results, 2-h plasma glucose levels during an OGTT were reported to be a better predictor than HbA1c for the development of cardiovascular incidents (33). Similarly, Ning et al. (34) reported a significant association between insulin resistance and increased risk of mortality from cardiovascular disease. However, this result was later disputed, citing the use of different diagnostic criteria for DM.

Determination of HbA1c is less time-consuming than the OGTT and is largely unaffected by fasting status. Furthermore, HbA1c is a better predictor of any future DM-related complications. Large epidemiologic studies have demonstrated a correlation between HbA1c levels and an increased risk of developing cardiovascular disorders, not only in patients with overt DM but also in those with IGT (35,36).

HbA1c may be used for making a diagnosis of T2DM in high-risk individuals. In a population where the prevalence of DM is constantly on the rise, there is a need for a test with high specificity to minimize false positivity.

One of the main limitations of our study could be the HbA1c assay used. In most of the previous studies, the HPLC (high-performance liquid chromatography) method was used for the determination of HbA1c, whereas the latex agglutination inhibition method was used in our study, mainly because of its availability in Turkish clinical laboratories.

Our study results suggest that HbA1c could be used to make a diagnosis of DM in Turkey. For HbA1c to overtake the OGTT as a reliable test would require the determination of an optimal cut-off value and the use of internationally standardized assays. Further studies on a larger scale are required in order to validate HbA1c assays as a reliable screening and diagnostic test for DM in Turkish individuals.

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