HbA1c measurement for the diagnosis of diabetes: is it enough?

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Abstract

Aim To analyse the performance of HbA1c in diagnosing Type 2 diabetes based on fasting plasma glucose and/or 2-h plasma glucose measurements after a 75-g oral glucose tolerance test.

Methods This is a study of diagnostic test accuracy in individuals referred to the Clinical Pathology Department for oral glucose tolerance testing. After fasting overnight, HbA1c, fasting plasma glucose and 2-h plasma glucose were measured. The receiver operating characteristic curve was used to evaluate the diagnostic performance of HbA1c.

Results Four hundred and ninety-eight subjects (195 male, mean age 56 years) were enrolled and 115 (23.1%) were diagnosed with diabetes according to glucose-based methods and only 56 (11.2%) individuals were identified by HbA1c > 6.5% (48 mmol/mol) (sensitivity 20.9%, specificity 95.3%). There is poor agreement between the newly recommended criterion and the current glucose-based diagnostic criteria (κ = 0.217; P < 0.001), probably because the diagnostic methods identify different populations of patients. Adding a glucose-based method into an algorithm, as proposed by the UK Department of Health, improved HbA1c performance.

Conclusions HbA1c > 6.5% (48 mmol/mol) showed limited sensitivity to diabetes diagnosis, although with high specificity. The results suggest that this cut-off point would not be enough to diagnose diabetes. Its use as the sole diabetes diagnostic test should be interpreted with caution to assure the correct classification of diabetic individuals.


Keywords diabetes, diagnosis, diagnostic tests, glycated hemoglobin

Introduction

Recently, an International Expert Committee, composed of members from the European Association for the Study of Diabetes, the International Diabetes Federation and the American Diabetes Association reported values of HbA1c ≥ 6.5% (48 mmol/mol) as the cut-off point for diagnosing diabetes [1]. The selection of this cut-off point was to emphasize specificity rather than sensitivity; however, the diagnostic performance of this specific cut-off point is not mentioned. Other studies had suggested lower HbA1c values, such as 5.8% (40 mmol/mol) and 6.0% (42 mmol/mol), for this purpose [2–5]. The United Kingdom Department of Health recommends the use of algorithms for diabetes screening in high-risk individuals that include traditional glucose diagnostic criteria or, alternatively, HbA1c measurements combined or not with glucose measurements. In non-symptomatic patients, a confirmed HbA1c ≥ 6.5% (48 mmol/mol) is enough to diagnose Type 2 diabetes [6]. However, this guideline also recommends that patients with HbA1c levels ≥ 6.0% (42 mmol/mol) and < 6.5% (48 mmol/mol) should undergo an oral glucose tolerance test to establish a diabetes diagnosis. No further test is required for those patients presenting with HbA1c levels < 6.0% (42 mmol/mol), but health lifestyle advice is given.

Until recently, the lack of HbA1c standardization prevented the use of HbA1c as part of a potential screening and diagnostic programme. The introduction of a new reference method improved HbA1c assay standardization worldwide and its role in the screening and diagnosis of Type 2 diabetes has been considered [7–9].

Taking into account that increased prevalence of retinopathy in different populations is already observed around HbA1c values of 6.0% (42 mmol/mol) [1], the risk
for cardiovascular disease is greater at the HbA1c level of 5.6–6.1% (38–43 mmol/mol) [10] and, bearing in mind that these individuals would benefit from early intervention, the aim of this report was to analyse the performance of the recommended HbA1c ≥ 6.5% (48 mmol/mol) value for the diagnosis of Type 2 diabetes [1,6] according to current diagnostic criteria based on fasting plasma glucose and/or 2-h plasma glucose after a 75-g oral glucose tolerance test [11,12].

**Research design and methods**

This is a study of diagnostic accuracy to evaluate the HbA1c test for diagnosis of diabetes (see also Supporting Information, Fig. S1). Conventional diagnostic tests of fasting plasma glucose ≥ 7.0 mmol/l and/or 2-h plasma glucose ≥ 11.1 mmol/l were considered to be the reference standard [11–13].

**Patients**

The study included individuals at high risk of developing or having diabetes being referred to the Clinical Pathology Department of the Hospital de Clinicas de Porto Alegre, between September 2008 and May 2009, to perform an oral glucose tolerance test. Porto Alegre is situated in Southern Brazil, where the majority of the population has European ancestry and approximately 86% of the population is of White ethnicity. All conditions known to interfere or lead to misinterpretation of HbA1c results were excluded [7,8]: patients with anaemia [14], estimated glomerular filtration rate < 60 ml min⁻¹ 1.73 m⁻² [15] and/or the presence of variant haemoglobin [16]. Patients signed an informed consent form and answered a standardized questionnaire. The protocol was approved by the Ethics Committee.

**Laboratory analysis**

After overnight fast, blood samples were drawn to determine HbA1c, lipid profile, creatinine and glucose levels, and oral glucose tolerance test was also performed according to World Health Organization recommendations [12]. Plasma glucose was determined by an enzymatic method and creatinine by Jaffé reaction (Modular P; Roche Diagnostics, Basel, Switzerland). HbA1c was determined by HPLC method (Tosoh 2.2 Plus HbA1c; Tosoh Corporation, Tokyo, Japan). This is a National Glycohemoglobin Standardization Program (NGSP)-certified method (http://www.ngsp.org/prog/index.html) and also International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) aligned [8]. The Clinical Pathology Department is a participant in and meets the standards of the HbA1c External Quality Assurance Program. Diagnosis of diabetes and impaired glucose tolerance were made according to current diagnostic criteria [11,12]. Impaired fasting glucose was classified as stated by American Diabetes Association criteria [11].

**Statistical analysis**

Data are expressed as mean and standard deviation when normally distributed and as median (range) for non-Gaussian variables. Student’s *t*-test, the Mann-Whitney *U*-test, McNemar test and kappa coefficient were used as appropriate. The receiver operating characteristic curve was used to analyse the performance of the HbA1c test to diagnose diabetes, using fasting plasma glucose and/or 2-h plasma glucose as reference diagnostic criteria. In addition, the UK Department of Health algorithm based on HbA1c results was also used to verify if an additional blood glucose measurement would improve the performance of HbA1c in diagnosing diabetes. A significance level of 5% was adopted.

**Results**

Four hundred and ninety-eight individuals were selected to participate in this study; 418 (83.8%) of those were of White ethnicity. The clinical and laboratory characteristics of all individuals are summarized in Table I. There was poor agreement between the diagnosis of diabetes using HbA1c ≥ 6.5% (48 mmol/mol) and any of the glycaemic tests (κ = 0.217; *P* < 0.001). According to the traditional criteria, 115 (23.1%) individuals were diagnosed with diabetes: 26 by fasting plasma glucose, 54 by 2-h plasma glucose and 35 using both tests. In contrast, although the HbA1c cut-off value of 6.5% (48 mmol/mol) has a specificity of 95.3%, this criterion identified only 56 (11.2%) individuals with diabetes; of those, 29 (25.2%) had the diagnosis established by current criteria: 22 by fasting plasma glucose and 2-h plasma glucose and seven by either fasting plasma glucose or 2-h plasma glucose. Therefore, the proportion of individuals with and without diabetes significantly differs between the glucose- and HbA1c-based methods (*P* < 0.001).

There were 86 individuals with a diagnosis established by current criteria and HbA1c < 6.5% (48 mmol/mol), corresponding to 74.8% of all diabetes cases. The sensitivity and specificity of the HbA1c cut-off value of ≥ 6.5% (48 mmol/mol), compared with the diagnosis of diabetes established by fasting plasma glucose and/or 2-h plasma glucose were 20.9 and 95.3%, respectively, resulting in a negative predictive value of 80.5%.

Based on receiver operating characteristic curve analysis, and considering fasting plasma glucose alone as the reference criterion, the cut-off point obtained by the point with the best equilibrium between sensitivity and specificity (100%-to-100% diagonal), a value of HbA1c of 6.0% (42 mmol/mol) was obtained. The sensitivity and specificity were 74.2 and 72.0%, respectively. The cut-off points of HbA1c of 7.0% (53 mmol/mol) and 8.0% (64 mmol/mol) presented specificities of 99.5 and 99.9%.

Alternatively, when fasting plasma glucose and/or 2-h plasma glucose and 2-h plasma glucose alone were considered as the reference criterion, an HbA1c level of 5.9% (41 mmol/mol) was
the equilibrium point for sensitivity and specificity (63.5/66.1 and 62.9/64.1%, respectively) (Fig. 1).

In our study, 167 patients had HbA1c ≥ 6.0% (42 mmol/mol). Sixty-five individuals were diagnosed with diabetes by the HbA1c ≥ 6.0% (42 mmol/mol) criterion and 50 were misclassified. There were 102 false positive results. Only nine individuals of those had normoglycaemia; however, 93 presented with hyperglycaemia (41 with impaired fasting glucose and 52 with impaired glucose tolerance). The sensitivity and specificity of the HbA1c cut-off value of ≥ 6.0% (42 mmol/mol) were 51.3 and 78.3%, respectively. The agreement between this cut-off point and current diagnostic criteria was also poor (κ = 0.274; P < 0.001).

Applying the UK Department of Health algorithm to verify if an additional blood glucose measurement would improve the performance of HbA1c in diagnosing diabetes, 82 individuals would be diagnosed with diabetes: in 56 individuals, HbA1c ≥ 6.5% (48 mmol/mol) and, in 26, the HbA1c level was between 6.0 and 6.5% (42–48 mmol/mol) and they had a positive oral glucose tolerance test. Fifty individuals with HbA1c < 6.0% (42 mmol/mol) and diabetes by the current criteria remained undiagnosed. There were 168 individuals presenting with HbA1c < 6.0% (42 mmol/mol) and an intermediate hyperglycaemia (50.7%). The sensitivity and specificity for this strategy were 47.8 and 92.9%, respectively. The agreement between this approach and current diagnostic criteria improved (κ = 0.416; P < 0.001).

**Discussion**

This study shows that there is poor agreement between the newly recommended criterion and the current glucose-based diagnostic criteria and, consequently, the proportion of patients diagnosed with diabetes by one or other method is significantly different.

Our results are in agreement with recent reports carried out in larger populations, which pointed out the poor diagnostic performance of HbA1c [17–19] and that it is not superior to fasting plasma glucose and/or 2-h plasma glucose [20]. The cut-off point of HbA1c ≥ 6.5% (48 mmol/mol) had a sensitivity of only 25% to diagnose diabetes. However, by adopting the cut-off of HbA1c ≥ 6.0% (42 mmol/mol), the sensitivity increased to 51.3%. Even so, approximately 50%
of patients with diabetes will not be identified. In addition, we observed an increase in false positive results using the cut-off point of HbA1c ≥ 6.0% (48 mmol/mol) 93% of the individuals misclassified presented some degree of glucose abnormality.

These findings suggest that the HbA1c method and the glucose-based methods identify different patient populations.

Several studies had suggested lower values than the present recommended values of HbA1c ≥ 6.5% (48 mmol/mol) to diagnose diabetes [3–5]. Besides, the cut-off value of HbA1c > 6.1% (43 mmol/mol) presented high sensitivity and specificity to screen for diabetes in high-risk individuals, and the combination of HbA1c and fasting plasma glucose improved the sensitivity for both tests alone [4]. Moreover, there is a continuous relationship between fasting plasma glucose and HbA1c and cardiovascular risk. It has been suggested that individuals with an HbA1c level of 5.6–6.1% (38–43 mmol/mol) and a fasting plasma glucose level of 5.6–6.3 mmol/l are at greatest risk for cardiovascular disease and should be targeted for further evaluation [10]. In a systematic review, the value of HbA1c > 6.1% (43 mmol/mol) was the recommended optimum cut-off point for the diagnosis of diabetes in most reviewed studies, although it is also stressed that cut-off points may be population-specific and may vary by race, age, gender and population prevalence of diabetes [5].

Alternatively, when the UK Department of Health algorithm [6] was applied and a glucose measurement was added to the HbA1c results between 6.0% (42 mmol/mol) and 6.5% (48 mmol/mol) to classify diabetic patients, the agreement between HbA1c and current diagnostic criteria improved. According to this algorithm, our data showed that individuals with HbA1c between 6.0% (42 mmol/mol) and 6.5% (48 mmol/mol) are likely to have an altered oral glucose tolerance test. A total of 111 individuals presented with HbA1c levels ≥ 6.0% (42 mmol/mol) and < 6.5% (48 mmol/mol) in our study, only nine had normoglycaemia and 102 presented some type of hyperglycaemia. The stigma and costs of a false positive diagnosis would be compensated for by identifying people with intermediate hyperglycaemia, considering the important clinical consequences of delaying the diagnosis [21,22]. However, 50 patients with HbA1c < 6.0% (42 mmol/mol) remained undiagnosed for diabetes by using this algorithm and would benefit from an additional test to establish their diagnosis. Additionally, there were 168 individuals presenting with HbA1c < 6.0% (42 mmol/mol) and an intermediate hyperglycaemia (50.7%).

Therefore, lowering the HbA1c cut-off level and adding a glucose-based method improved the HbA1c performance in diagnosing diabetes, suggesting that each method identifies different patient populations.

In conclusion, HbA1c ≥ 6.5% (48 mmol/mol) showed high specificity but limited sensitivity to a diabetes diagnosis. The results suggest that the cut-off point of ≥ 6.5% would not be enough to diagnose diabetes. Its use as the sole diabetes diagnostic test should be interpreted with caution to assure the correct classification of diabetic individuals.

Competing interests

Nothing to declare.

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