Should haemoglobin A_{1c} be used for the diagnosis of diabetes mellitus in South Africa?

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Keywords: diabetes, glycated haemoglobin, fasting plasma glucose, oral glucose tolerance test

Abstract

Diabetes is an important medical problem in sub-Saharan Africa. It has traditionally been diagnosed by means of fasting plasma glucose, random blood glucose or an oral glucose tolerance test. Each of these has limitations and, in 2009, an expert committee of the American Diabetes Association recommended using the haemoglobin A_{1c} (HbA_{1c}) to diagnose diabetes mellitus.[1] The aim of this paper is to analyse the advantages and disadvantages of using HbA_{1c} as a diagnostic method for diabetes, and its applicability in the South African setting.

Ⓟ **Peer reviewed. (Submitted: 2011-04-05. Accepted: 2011-08-08.)** **JEMDSA 2011;16(3):122-127**

Introduction

Over the past two decades, type 2 diabetes has emerged as an important medical problem in sub-Saharan Africa. Estimates by the International Diabetes Federation suggest that the largest increase in the prevalence of diabetes is expected to occur in the developing world, including Africa. However, there is a paucity of prevalence data from South Africa and the rates published in the few studies available vary depending on the populations studied, as well as the method used in the diagnosis.

Before 2009, the only acceptable tests for the diagnosis of diabetes were based on blood glucose values: either a fasting blood glucose (FPG) of at least 7 mmol/l or a random blood glucose of at least 11.1 mmol/l in a patient with symptoms of diabetes, or a blood glucose of at least 11.1 mmol/l two hours after an oral glucose tolerance test (OGTT). In 2009 an expert committee of the American Diabetes Association (ADA) recommended the use of HbA_{1c} to diagnose diabetes.[1] In 2010 the ADA adopted the proposal as part of the diagnostic criteria of diabetes, using a cut-off of equal to or greater than 6.5%. They modified the criteria for impaired fasting glucose and impaired glucose tolerance to include individuals with an HbA_{1c} ranging from 5.7 to 6.4%, and recommended that the diagnosis should be confirmed with a repeat HbA_{1c} test, unless there are clinical symptoms and blood glucose levels equal to or greater than 11.1 mmol/l. Their recommendations also state that the HbA_{1c} must be measured in an accredited laboratory and that the different methods for diagnosing diabetes should not be used together, because of the lack of agreement between them.[2]

In 2009 the World Health Organization (WHO) conducted a systematic review of the use of HbA_{1c} to diagnose diabetes, and made similar recommendations. They recommend that the HbA_{1c} be used for the diagnosis of diabetes using a cut-off value of 6.5%, provided that stringent quality assurance tests are in place and assays are standardised to criteria aligned to international reference values, and that there are no conditions present which preclude the accurate measurement of HbA_{1c}.[3] The WHO also recommended that long-term prospective studies are carried out in various ethnic groups to establish the precise glucose and HbA_{1c} levels predictive of microvascular and macrovascular complications. The WHO recommendation states that in an asymptomatic individual diagnosis should be confirmed with an additional test. It also cautions against the use of point-of-care devices, except where no other technology is available, and provided that stringent quality assurance programmes are in place.

The aim of this paper is to analyse the advantages and disadvantages of using HbA_{1c} to diagnose diabetes in a multiracial society such as South Africa

Evidence for the use of HbA_{1c}

Glycated haemoglobin reflects the average endogenous exposure to glucose, including postprandial spikes, and has low intra-individual variability, particularly in individuals without diabetes. It is these characteristics that may

contribute to its apparent superiority over fasting glucose in risk stratification for cardiovascular disease.

The evidence for the use of HbA_{1c} for diagnosing diabetes is based on the examination of three cross-sectional epidemiological studies that included an Egyptian population (n = 1018), a Pima Indian group (n = 960) and the United States National Health and Nutrition Survey (NHANES, n = 2821). Each of these studies assessed FPG, two-hour glucose and HbA_{1c}, and also measured the prevalence of retinopathy as assessed by fundus photography or direct ophthalmoscopy. The HbA_{1c} values at which retinopathy increased in prevalence were between 6 and 7% and were similar among the three groups.[1] It is important to note that, in at least two of these studies, the populations studied were homogenous. A systematic review of nine studies performed in Asian or European populations compared HbA_{1c} with FPG for the diagnosis of diabetes and used WHO criteria as the gold standard. They reported that HbA_{1c} and FPG were equally effective tools for the diagnosis of diabetes. An HbA_{1c} equal to or greater than 6.1% had a sensitivity of 78 to 81% and a specificity of 79 to 84% for the diagnosis of type 2 diabetes. When they used an FPG value of 6.1 mmol/l as cut-off point, the sensitivity ranged between 48 and 64% and the specificity ranged from 94 to 98%.[4] In a population-based study from Singapore, the investigators showed that increasing HbA_{1c} was associated with all microvascular complications, and that the optimal cut-off point for detecting mild and moderate retinopathy was between 6.6% and 7% respectively, with the prevalence of retinopathy being less than 1% below these cut-off points.[5] The Atherosclerosis Risk in Communities (ARIC) study is a community-based prospective cohort of middle-aged adults from the USA. In this study, data from more than 14 000 black and white adults demonstrated that increasing HbA_{1c} levels were associated with increasing values of FPG, but were more strongly associated with risk of cardiovascular disease and death from any cause compared with FPG.[6]

Comparison of HbA_{1c} with other methods of diagnosing diabetes

Oral glucose tolerance test

OGTT is recognised as the gold standard in the diagnosis of diabetes and is a sensitive marker of impaired glucose tolerance. However, it requires stringent conditions, such as the ingestion of at least 150 g of carbohydrate per day for at least three days prior to the test, overnight fasting of at least 10 hours, and preventing the patient from walking around during the test.[7] These conditions not only influence the test results, but often make the test impractical. The OGTT also shows much greater intra-individual variability (16.7%) than the FPG or HbA_{1c}.[8] It is because of these limitations that the ADA recommended FPG as the preferred glucose-based diagnostic test for diabetes.[9]

Fasting plasma glucose

Small increases in blood glucose substantially increase the risk of diabetes, but glucose measurement is affected by several preanalytical and analytical variables that make it difficult to apply these epidemiological principles to individual patients. Patients are seen by their physicians at any time of day and in an uncertain state of fasting. Fasting glucose levels show significant diurnal variation, which could impact on the diagnosis of diabetes.[10]

To reduce glycolysis, glucose is collected in fluoride tubes, after which it should be placed on ice and the plasma should be separated from the cells promptly.[11] In spite of these precautions, the rates of decrease in glucose in the first hour after collection in tubes with and without fluoride are virtually identical, and the decrease in glucose after two hours in a fluoride tube can exceed 0.5 mmol/l.[12]

Another problem with the use of FPG alone for the diagnosis of diabetes is that, in epidemiological surveys, it results in a lower disease prevalence compared to the use of the OGTT, and it does not pick up those individuals with impaired glucose tolerance. Motala et al investigated diabetes and other disorders of glycaemia in rural KwaZulu-Natal and showed that if FPG alone was used, the prevalence of diabetes would be 36% lower than when a full OGTT was performed. If an OGTT was not performed, none of those subjects with impaired glucose tolerance would be detected.[13] Soma and Rheeder noted in a group of 120 subjects admitted for elective coronary angiography that nine out of 14 subjects would not have been detected as being diabetic if only an FPG level had been measured, as opposed to a full OGTT.[14]

Plasma is the preferred sample type for glucose measurement in the laboratory and there is variability when sample types differ. Whole-blood glucose concentrations are lower than plasma concentrations because of the lower water content of red blood cells, while plasma glucose concentrations have been reported to be lower, higher or the same as those in serum.[15-18]

HbA_{1c}, on the other hand, does not require an overnight fast and it does not require morning blood collection. HbA_{1c} is also reported to be relatively stable and shows less intra-individual variability than FPG or two-hour glucose levels. This was illustrated by Selvin et al, who looked at short-term inter-individual variability of FPG and two-hour glucose and HbA_{1c} in 685 participants without diagnosed diabetes, and showed high variability of both FPG and two-hour glucose levels relative to HbA_{1c} levels.[8]

The case against HbA_{1c}

Despite its greater clinical convenience, preanalytical and biological stability, and assay standardisation, HbA_{1c} has several limitations that preclude its use for the diagnosis of diabetes in South Africa at this time.

Methods of measuring HbA_{1c}

Laboratories use a wide variety of analytical techniques to separate and then quantify HbA_{1c}, based on differences in either molecular structure or molecular charge.

HbA_{1c} is less positively charged at neutral pH than pyridoxylated normal adult human haemoglobin (HbAo), and this is the basis for the high-performance liquid chromatography (HPLC) method. Spectrophotometric analysis of the various eluants provides the percentage of each haemoglobin species in the sample. This method is rapid and has very good precision. In South Africa it is utilised in some private laboratories, as well as in some of the large academic hospitals. The separation of total glycated haemoglobin or HbA_{1c} based on molecular structure can be achieved either by immunoassay or by boronate affinity chromatography. Immunoassays in particular may be affected by haemoglobinopathies. Several South African laboratories use immunoassay-based methods to separate glycated haemoglobin from the nonglycated haemoglobin (Cummins R, Fedler C, Zemlin A, personal communication).

Owing to simplicity of use, small sample volume requirements and portability, there is interest in the use of point-of-care devices for the measurement of HbA_{1c}. While these are acceptable for the monitoring of glycaemic control in diabetic patients, the ADA cautions that point-of-care devices should not be used for the diagnosis of diabetes. An evaluation of eight NGSP (previously the National Glycohemoglobin Standardization Program) -certified HbA_{1c} point-of-care instruments showed that only two of these instruments met the acceptance criteria of having a coefficient of variation (cv) of less than 3%, and all the instruments showed significant differences in analytical performance between different reagent lot numbers.[19] In addition, point-of-care tests cost more than laboratory-based methods (George J, personal observation). There is no objective information concerning their performance in the hands of those who use them, and proficiency testing might not be carried out.

Standardisation and quality assurance

The ADA endorsement of HbA_{1c} for the diagnosis of diabetes is based partly on the fact that assays are now highly standardised.[20] This process began in 1993, when the American Association for Clinical Chemistry (AACC) established a Glycohaemoglobin Standardization Subcommittee to formulate a strategy to harmonise glycated haemoglobin results. The goal of the NGSP was to standardise HbA_{1c} results so that values reported by clinical laboratories were comparable to those reported by the Diabetes Control and Complications Trial (DCCT). The rationale for this was that the DCCT showed that risks for complications in patients with diabetes were directly related to glycaemic control as measured by HbA_{1c}. In 2001, a reference method and reference material was developed by a working group of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and this method is used by manufacturers to standardise assays.[21] When the results of pooled blood samples in the IFCC and NGSP networks were compared they showed that the relationship is linear: NGSP % = (0.915 × IFCC %) + 2.15. While there is good linear correlation between the NGSP and IFCC results, the absolute HbA_{1c} values differ by 1.5 to 2%. The IFCC wanted HbA_{1c} reported as mmol/mol, but because of concerns about possible confusion arising if patients' HbA_{1c} values were changed, agreement was reached between the IFCC and major diabetes organisations to report IFCC HbA_{1c} results (mmol/mol) as the equivalent NGSP DCCT-aligned result (percentage based on the equation).

All methods used by the major laboratories in South Africa are NGSP certified. This means that, for a panel of 40 samples analysed in duplicate under ideal conditions, the 95% confidence interval of the difference between the method being tested and an NGSP reference laboratory would be within ± 0.75% HbA_{1c}. Method certification is performed by the manufacturer. Laboratories can obtain documentation from the manufacturer on their system's performance and traceability to DCCT, and should do so, but this does not guarantee accuracy and precision in the clinical laboratory. Laboratories should therefore participate in external proficiency testing programmes using whole-blood quality control material. There is no national quality assurance programme for HbA_{1c} in South Africa at this time, nor are there data on methodologies currently in use, or on participation and performance in proficiency testing. Until laboratories here demonstrate that their assays are aligned to international reference values and proper quality assurance criteria are adhered to, and that these laboratories are accredited as stipulated by both the ADA and the WHO, HbA_{1c} should not be used for the diagnosis of diabetes.

Ethnicity

A number of studies have shown that the cut-off of 6.5%, as proposed by the ADA, is not optimal for the diagnosis of diabetes in some ethnic groups. In an Indian population a cut-off equal to or greater than 6.1% was shown to have

better diagnostic performance than a cut-off of 6.5%.[22] It appears that people of African descent have higher levels of glycated haemoglobin across the full spectrum of glycaemia.[23] Analysis of data from over 2 000 nondiabetic individuals in the NHANES III population showed that the HbA_{1c} level increased with age and with ethnicity. In non-Hispanic blacks, and using HbA_{1c} levels of 6.5 to 6.9%, 87% of those over the age of 64 years would not have diabetes by FPG/OGTT criteria.[24] Jorgensen et al assessed whether ethnicity modified the association between glucose levels and HbA_{1c} among Greenland Inuit, Inuit immigrants in Denmark and the general Danish population.[25] They looked at OGTT and HbA_{1c}. All three groups had an increase in prevalence when HbA_{1c} was used for diagnosis. However, this was most dramatic for the Greenland Inuit, with a jump from 11.2% (OGTT) to 31.7% (HbA_{1c}). Another drawback of the use of HbA_{1c} at this time is that studies comparing FPG- and HbA_{1c}-based diagnosis often produce discordant results. Analyses of data from nondiabetic Koreans showed that 31.6% of the subjects would have been classified as having diabetes based on FPG, while only 23.5% would have been classified as having diabetes using HbA_{1c} value of 6.5%.[26]

What these studies tell us is that, in some population groups, using FPG to diagnose diabetes results in an increased prevalence when compared to the use of HbA_{1c}, while the opposite is observed in other populations. The selection of a diagnostic threshold is usually made by means of the receiver operating characteristics curve (ROC) approach, followed by its validation in the target population using likelihood ratios. This evidence is lacking for South Africa.

In addition to ethnicity, other factors that affect HbA_{1c} levels include age, anaemia, human immunodeficiency virus (HIV) status, renal failure, erythrocyte biochemistry and haemoglobinopathies.

Ageing

Most data on the use of HbA_{1c} for the diagnosis of diabetes are based on studies carried out in adults. A cross-sectional analysis of data from the NHANES III group demonstrated a consistent increase in HbA_{1c} with age.[27] There are limited data from children. One recent study carried out on a multiethnic cohort of children and adolescents suggests that a cut-off value of 6.5% underestimates the prevalence of diabetes in children.[28] Thirty per cent of the population in South Africa is under the age of 15 and 8% is over the age of 60, and the use of the prescribed cut-off of 6.5% may be inaccurate in both these groups.[29] At present, neither the WHO nor the ADA advocates the use of age-appropriate reference ranges.

Renal failure

There are few data on the prevalence of chronic kidney disease in Africa, but the general impression is that it is three to four times more frequent than in more developed countries.[30] Chronic kidney disease may falsely increase or decrease HbA_{1c} levels. Increased values can arise from increased blood urea levels, which cause the formation of carbamylated haemoglobin and which then results in overestimation of haemoglobin using electrical charge-based assays.[31] These patients often have a shortened red blood cell life span and this would potentially reduce HbA_{1c} levels.[32]

HIV infection

A report from the Women's Interagency HIV study, which looked at FPG and HbA_{1c} in HIV-infected and noninfected women, showed that HbA_{1c} levels are slightly lower in HIV-infected women than in noninfected women; this was mainly due to higher mean corpuscular volumes.[22] In the proceedings from the Conference on Retroviruses and Opportunistic Infections 2011, investigators showed that using an HbA_{1c} cut-off of 6.5% would have diagnosed only nine out of 22 patients not previously known to be diabetic, and that in this small group of subjects the optimal cut-off for diagnosis was 5.8%. Of interest was that the use of antiretroviral drugs showed a variable influence on the test characteristics of HbA_{1c}, with protease inhibitors leading to an underestimation of HbA_{1c} levels and non-nucleoside reverse transcriptase inhibitors, particularly efavirenz, overestimating HbA_{1c} levels.[33] This has major implications for our population, which has a high prevalence of HIV infection, and the use of efavirenz in first-line drug treatment.

Glycation gap and erythrocyte biochemistry

One of the explanations put forward for the observed differences in sensitivity and specificity of Hb_{A1c} as a diagnostic tool is the differences in rates of glycation of haemoglobin.[34] Yudkin et al introduced the terms "high glycators" and "low glycators" to describe individuals with higher or lower HbA_{1c} levels relative to their glucose.[35] Studies comparing intracellular glycation, as assessed by HbA_{1c}, with extracellular glycation, as assessed by glycated serum proteins, have shown very strong within-person correlation between HbA_{1c} and fructosamine, but substantial between-person variation in the relationship between fructosamine and HbA_{1c}.[36] This tells us that that there are large inter-individual differences between HbA_{1c} and glucose. Biochemical differences in glucose transport across red cell membranes, subclinical variation in erythrocyte survival, and inherited differences in glycation rates all contribute to the glycation gap.[37-39]

Anaemia, iron deficiency and haemoglobinopathies

Conditions that alter red cell life span alter HbA_{1c} concentrations, with conditions that shorten red cell survival, such as haemolysis, decreasing HbA_{1c}, and those disease states that prolong red cell survival increasing HbA_{1c}.[37] Sickle-cell haemoglobin (HbS) has a valine for glutamic acid substitution at position 6 of the β chain. HbC is a variant mutation at the same site as the sickle cell mutation. Because the S and C variants are close to the N terminus of the β chain, some immunoassays are affected by the presence of these variants. These variants do affect the ionic charge of the haemoglobin molecule, and this may interfere with ion exchange methods. However, careful inspection of chromatograms should identify the presence of abnormal haemoglobin variants and alternate methods may be used.

No whole-blood method is suitable for the assessment of glycaemic control in patients homozygous for HbS, HbC or HbSC. The prevalence of sickle cell disease in indigenous South Africans is low, but can be very high in populations from the malaria-endemic regions of Africa.[40]

Iron deficiency has been shown to increase HbA_{1c} by up to 2%.[41] This is reversed by iron supplementation.[42] The prevalence of iron deficiency in an urban South African female population despite iron fortification was estimated to be about 10%,[43] while the prevalence in rural populations might be higher, at between 18% and 25%.[44,45] In view of these patient variables it is likely that the use of HbA_{1c} at a cut-off level of 6.5% will lead to overdiagnosis in the elderly, those with iron deficiency and some rapid glycators, while those with renal failure, haemoglobinopathies and slow glycators will be underdiagnosed.

Given the high prevalence of HIV infection in our population, and the frequency with which subjects with diabetes have other medical illnesses, the likelihood that such factors may alter HbA1c is high. Thus it is imperative that large epidemiological studies be undertaken in such patient groups in South Africa to assess the diagnostic accuracy of HbA_{1c} measurement.

Costs and availability

The cost of HbA1c is more than double that of plasma glucose, but is cheaper than the cost of the OGTT, which includes the cost of a glucose load and two glucose measurements. However, it is probable that other tests will be required in a significant proportion of cases and this will increase the economic burden on the health system. Currently there are no reliable data on the availability of the HbA_{1c} test in South Africa.

Conclusion

In conclusion, high HbA_{1c} levels are clearly associated with incident microvascular complications in cohorts of previously diagnosed diabetics. The available evidence supports the role of HbA_{1c} as a marker of glycaemic control and as a prognostic marker for future complications. However, there is insufficient evidence from South Africa at this time to recommend it as the method of choice for diagnosis. Before it can be recommended for the diagnosis of diabetes in South Africa, it is important to assess the methodologies currently in use and to ensure that they are reliable and traceable to the DCCT method. It is also important that laboratories are accredited and participate in external proficiency programmes. We should work towards the use of NGSP-certified methods for all diagnostic laboratories, and a national quality control programme for HbA_{1c} measurement.

Appropriate cut-off levels for the population should be determined, based on the association between HbA_{1c}, FPG, two-hour glucose levels and microvascular complications. Furthermore, the effects of common diseases, such as HIV and malaria, and the effects of antiretroviral therapy on the utility of HbA_{1c} for the diagnosis of diabetes must be assessed.

Wild et al estimated that, in 2000, more than seven million people in sub-Saharan Africa had diabetes, with a projected increase to over 17 million by 2030. These projections do not take into account the effect of urbanisation and associated increases in rates of obesity across much of urban Africa.[46] Prospective evaluations should be carried out to assess the clinical and economic consequences of modification of the current diagnostic criteria. In the meantime, we should ensure that the majority of the population has access to plasma glucose and the OGTT for the diagnosis of diabetes.

Presently, the HbA_{1c} should continue to be used as a means of monitoring diabetes control in South Africa, but not for its diagnosis.

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