

HbA_{1c} as a marker of prediabetes: A reliable screening tool or not?

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Abstract

Increasing global prevalence of type 2 diabetes (T2D) has resulted in concerted efforts to improve predictors for development of this obesity-related disorder. Establishing markers that identify prediabetes, an intermediary state of glycaemia above that of healthy individuals but below frank T2D, is an important focus. International cut offs have long been based on the 2 h WHO-defined oral glucose tolerance test (OGTT), but more recent use of the quicker and cheaper marker of glycated haemoglobin (HbA_{1c}) has become widespread in clinical practice and public health. The definition of people with prediabetes in turn has expanded from those with impaired glucose tolerance (IGT) to include individuals with impaired fasting glucose (IFG) and/or raised HbA_{1c}. Whilst HbA_{1c} has been recommended since 2010 for both T2D and prediabetes screening, concerns have been raised over validity particularly for identifying those who will later develop T2D. Depending on criteria, HbA_{1c} may identify only 50% with abnormal OGTT or misclassify those with normal physiology. Models predicting average time intervals for progression to T2D from prediabetes are commonly limited by ethnic, racial and gender differences, and different criteria further result in variable estimates of prediabetes prevalence and impact those eligible for lifestyle interventions. Whilst HbA_{1c} may provide a good marker of frank T2D, some recommend its use in prediabetes only in conjunction with fasting plasma glucose (FPG). This review updates current opinion on HbA_{1c} as an effective screening method for categorising high-risk prediabetic individuals and those requiring fast track into lifestyle modification programs.

Keywords: Glycated haemoglobin, HbA_{1c}, Type 2 diabetes, Prediabetes, Fasting plasma glucose, Oral glucose tolerance test, Impaired glucose tolerance, Impaired fasting glucose.

Abbreviations:

WHO: World Health Organization; ADA: American Diabetes Association; HbA_{1c}: Glycated Haemoglobin; IGT: Impaired Glucose Tolerance; IFG: Impaired Fasting Glucose; T2d: Type 2 Diabetes; OGTT: Impaired Glucose Tolerance Test

Introduction

Type 2 diabetes (T2D) is becoming an increasingly common disease with incidence rates that are rising rapidly in parallel with the rising global prevalence of overweight and obesity [1]. In 1994 approximately 1 million people globally were reported with T2D, which increased to 382 million in 2013, and now with a projected increase to 592 million over the next 20 years [2]. High T2D prevalence results in both decreased quality of life for the individual and increased government health care costs resulting from increased morbidity, largely a result of macro and microvascular conditions caused by long-term elevations in peripheral blood glucose. A disease long known

in those who are 'overweight and over forty' it is gradually becoming a disease of younger adults, adolescents and even children as lifestyle changes lead to weight gain and increased adiposity [3]. Those who have high levels of central adiposity are at particular risk of T2D, with abdominal obesity strongly associated with important changes in body composition including lipid overspill/infiltration into critical organs such as the pancreas and liver [4].

Whilst it has long been shown that weight loss can reverse the high blood glucose levels by which T2D is defined [5], and thus halt the worsening of T2D-driven macro and microvascular complications, successful long term weight loss is difficult to achieve and rarely successful for most overweight individuals other than those undergoing invasive bariatric procedures [6,7]. Industrialised environments have led to a major change in diet and physical activity over the past 40-50 years that strongly promotes weight gain and prevents weight loss.

In addition, even some individuals who appear outwardly lean and healthy may have increased T2D risk as a result of lipid infiltration into organs, an increasing common phenomenon for example in Asian populations, and which has been termed the ‘thin on the outside fat on the inside’ (TOFI) profile [8]. Identifying who in our community is at increased risk of T2D must be an important part of any disease prevention strategy. Developing simple public health screening methods that effectively identify individuals who are at a greater risk of T2D, is extremely important as it allows timely intervention both to delay and/or prevent progression. Measurement of glycated haemoglobin (HbA_{1c}), a longer term marker of high blood glucose concentration, is a simple and cheap method that has been adopted by the American Diabetes Association (ADA) within the past 10 years [9] for diagnosis of T2D. Glycation of haemoglobin (Hb) occurs throughout the lifespan of a red blood cell which is typically 120 days, which means the relative proportion of HbA_{1c} at any one time depends on the mean circulating blood glucose level over that 3 month period. Use of HbA_{1c} as a screening marker has rapidly been adopted in clinical practice by a growing number of countries where it may provide an excellent cost efficient approach to T2D screening providing it is shown to have adequate sensitivity and specificity. Comparison of costs from the National Institute for Health and Clinical Excellence (NICE) UK, for example, showed the indicative cost of HbA_{1c} test to be half that of an OGTT (GB£4.04/US\$5.05 vs. GB£7.48/US \$9.34) [10] when widespread testing was adopted in 2011 [11]. Whilst there is evidence that it may be a good marker of frank T2D, there is considerably more controversy as to whether it may also correctly identify those prediabetic individuals with increased future risk of T2D but as yet without the full disease [12].

Prediabetes

A high risk profile for T2D has been officially recognized for many years by varied names, such as ‘borderline diabetes’ [13] and is primarily based on an individual’s glycaemic state. More recently the term ‘prediabetes’ or ‘intermediate hyperglycaemia’ has evolved [14]. Irrespective of terminology, all are defined by glucose parameters that are higher than normal physiology but lower than the threshold limits set for diagnosis of T2D. However screening has been largely dependent on the criteria that has been used to define this intermediary glycaemic state, and which has varied considerably from year to year (Table 1), in turn altering the cohort of individuals who are considered at increased risk of T2D and hence most in need of prevention and/or treatment. To better predict when this increased risk begins, the World Health Organization (WHO) developed criterion to demarcate and classify individuals with newly identified intermediate hyperglycaemia or prediabetes into two early stages of abnormal glucose homeostasis; [i] impaired fasting glucose (IFG) where an individual has poor glucose regulation and raised blood glucose even when overnight fasted and [ii] impaired glucose tolerance (IGT) where an individual fails to respond to glucose consumed as part of a meal leading to raised postprandial blood glucose [15] (Table 1). Both result in high circulating levels of glucose, which in turn may adversely alter key proteins through glycation (e.g. HbA_{1c}), yet the IFG and IGT population may comprise quite different individuals or cohorts with quite different aetiology of prediabetes and little or no overlap. Whether HbA_{1c} can adequately predict risk of T2D in the absence of more detailed blood glucose assessment remains a topic of considerable debate.

Table 1. Diagnostic criteria for prediabetes.

Venous plasma glucose				
International Panel	Fasting (mmol/L)	2 h post load/OGTT* (mmol/L)	Classification/ Subcategory	HbA _{1c} % (mmol/mol)
WHO, 1965		7.1-8.2		-
WHO,1980	<8.0	≥ 8.0 and <11		-
WHO, 1985	<7.8	≥ 7.8 and <11.1		-
WHO, 1999	<7.0	≥ 7.8 and <11.1	IGT	-
and 2006 (most recent)	≥ 6.1 and <7.0	<7.8 (if measured*)	IFG	
ADA, 1997	<7.0	≥ 7.8 and <11.1	IGT	-
	6.1-6.9	N/A	IFG	
ADA, 2003	<7.0	7.8-11.0 (if measured*)	IGT	-
	5.6-6.9†	N/A	IFG	
ADA, 2010 (most recent)	<7.0	7.8-11.0	IGT	5.7-6.4 (39-47)
	5.6-6.9 †	N/A	IFG	

WHO – World Health Organization [96-99]; ADA – American Diabetes Association [9, 21, 99]; HbA_{1c} – Glycated

haemoglobin; IGT – Impaired glucose tolerance; IFG – Impaired fasting glucose.

*Measurement recommended to exclude diabetes or IGT, † 2-hr post load glucose measurement not recommended.

To consider in more detail, IFG reflects increased gluconeogenesis and increased hepatic glucose output into peripheral circulation even in the absence of a meal, i.e., when no dietary glucose has been consumed. This is determined by measuring fasting plasma glucose (FPG), following an 8-12 h overnight fast [16]. Conversely IGT is indicative of inadequate postprandial insulin secretion from the pancreas after glucose has been consumed as a bolus or within a meal, and which results in poor insulin-mediated glucose disposal from circulation into tissues. It is diagnosed using a standard WHO 75 g oral glucose tolerance test (OGTT) where glucose levels are assessed over a 2 h period following a fixed glucose load [17]. Although pancreatic β -cell dysfunction and hence inadequate insulin secretion is present in individuals diagnosed with both isolated IFG and isolated IGT and both groups also experience insulin resistance, the site of the resistance is quite different. While individuals with IFG have severe hepatic insulin resistance with normal or near normal insulin sensitivity in skeletal muscle [18,19], those with IGT have severe insulin resistance in skeletal muscle with only a modest increase in hepatic insulin resistance [18, 20].

The WHO recommended cut offs based on these parameters received support from the ADA who in 2003 further lowered the cut points for diagnosis of IFG from 6.1-6.9 mmol/L to 5.6-6.9 mmol/L [9,21] (Table 1). Whilst this greatly expanded the population size considered to be at risk of later T2D, the term prediabetes has received criticism as not all diagnosed individuals progress to frank T2D and implies that no intervention may be necessary due to the absence of full blown disease. Annual incidence of progression to T2D in individuals with isolated IGT (4-6%) or isolated IFG (6-9%) have been reported to be lower than in those with both IFG and IGT (15-19%) [22]. Progression estimates in larger prospective studies have reported similar annualised incidence of risk using isolated IFG [23,24] or both [25]. Annually approximately 5-10% prediabetic individuals progress to T2D with rates varying depending on population characteristics as well as the criteria and cut offs used for defining prediabetes [26,27].

Whilst both FPG and OGTT have been relied upon to successfully screen 'at risk' individuals several key issues limit utility in routine practice. Both tests require precise preparation, including restricted diet and exercise on the day prior, to improve accuracy, and individuals must be fasted overnight (minimum 8 h). Samples require immediate measurement and centrifugation within 30 min following collection. Owing to the inconvenience of measuring FPG or performing an OGTT and the day to day variability in blood glucose levels, the ADA recommended in 2010 that the screening and diagnostic accuracy be improved based on measures of glycosylated or glycated haemoglobin, HbA_{1c} (Table 1) [9]. The underlying premise for the addition of HbA_{1c} was that it would capture chronic exposure to both basal and postprandial hyperglycaemia in individuals over a longer period and therefore could reflect a combination of the pathophysiological defects underlying IFG and IGT over time.

HbA_{1c} – A More Favourable Marker for Diagnosis of Prediabetes and T2D?

HbA_{1c} was first described in 1969 by Rahbar et al. [28] and due to the biochemistry of the protein considered to be a more reliable long term marker of glycaemic control [29], with elevated levels positively correlated to the increased risk of T2D [30,31]. Importantly, measures of HbA_{1c} provide a weighted average of blood glucose for the lifespan of the red blood cell, typically 2-3 months, with the last month contributing ~50% of the result [32]. The Hb molecule is a tetramer and is formed of two alpha and two beta globin chains. Exposure to high concentrations of blood glucose results in the non-enzymatic glycation of Hb at various sites of the molecule, and when reversibly glycated at the N-terminal valine residue of the beta chain leads to the formation of HbA_{1c} [33]. Logistically, the test can be performed at any time of the day and does not require patient preparation or an overnight fast. The marker reliably measures degree of glucose exposure over time [34,35] and is better related to the risk of micro and macro vascular complications [36-38]. Unlike a simple FPG assessment, HbA_{1c} levels also represent postprandial glucose intolerance and therefore could be more efficacious in screening overweight prediabetic individuals with glucose abnormalities [39]. Furthermore, HbA_{1c} measures are reported to be relatively stable and have greater reproducibility and lower variability between and within individuals than that observed for glucose measurements [40]. The day to day within person variation in HbA_{1c} is reported to be <2% in comparison to 12-15% for FPG [41,42].

HbA_{1c} Cut Points – Where to Set the Threshold Values

Despite the favourable attributes of HbA_{1c} measurements there have been several issues with the use of this marker that may preclude meaningful comparisons between published data sets. One of the key limitations still under debate by Expert Committees is the threshold values for diagnosis of prediabetes. Ideally selection of diagnostic HbA_{1c} cut points for prediabetes ought to be based on evidence that intervention, when applied to the 'at risk' group, results not only in the prevention of T2D but also later complications. However trials addressing these issues have reached no consensus regarding optimal cut points for HbA_{1c}. In the absence of such data, Expert Committees rely on information about the shape of risk curves for complications such as retinopathy; much like that for the cut offs for T2D. WHO suggested HbA_{1c} cut offs for prediabetes be set at 6.0–6.4% (42–46 mmol/mol). This has latterly been revised by the ADA and lowered to 5.7–6.4% (39–46 mmol/mol) [14], a decision however that has not been endorsed by any other group. These limits are in agreement with a recent systematic review of 16 prospective studies that show that HbA_{1c} values between 5.5–6.5% were associated with a significant increased risk for developing T2D [43]. An argument can however be made against the lowered threshold as it may cause an imperfect overlap to create a large, poorly characterised and heterogeneous category of glucose

intolerance resulting in an increased prevalence of prediabetes [44-46].

The optimal thresholds of HbA_{1c} for the diagnosis of frank T2D based on incidence of retinopathy, a common comorbidity of T2D caused by hyperglycaemia-driven changes in microvascular blood vessels of the eye [47-49], are also under careful scrutiny. While the ADA HbA_{1c} cut point of 6.5% (48 mmol/mol) has been shown to represent the same threshold for increased prevalence of retinopathy as FPG \geq 7.0 mmol/L and 2 h plasma glucose levels \geq 11.1 mmol/L [14], only a single study has validated the inflection point of HbA_{1c} \geq 6.5% for increased incidence of retinopathy [50]. This result was not supported by other studies such as that in Pima Indians [51] where the threshold HbA_{1c} for retinopathy was \geq 6.9% [52 mmol/mol], while in the European DESIR (Data from an Epidemiological study on the Insulin Resistance Syndrome) study [52] the positive predictive values for retinopathy increased sharply at HbA_{1c} 6.0% (42 mmol/mol). Again, in the Hoorn Study [53] a clear cut off point could not be identified due to the wide range of HbA_{1c} (5.8–13.1%) that described 21.1% incidence of retinopathy in the Dutch population. Similarly, a 14 year follow up study by Selvin *et al.* [54] did not find a HbA_{1c} threshold for microvascular outcomes before or after adjusting for covariates. Based on these longitudinal studies the HbA_{1c} cut point of 6.5% for diagnosis of T2D also appears to require further investigation.

Biological Variability in Cut Points – Do Threshold Values Apply to All?

Whilst HbA_{1c} is a long term robust marker for determining variability in glycaemic status the observed discrepancies in reported literature requires a better understanding of biochemical changes that may impact results. HbA_{1c} threshold limits may be affected by ethnic and racial differences in red cell turnover or Hb glycation that account for variability in HbA_{1c} measures between populations. This is termed the glycation gap or glycation index [55]. A systematic qualitative review of HbA_{1c} levels across racial and ethnic groups in the US, has reported that those of African-American descent had the highest level of HbA_{1c} in comparison to Hispanics, and that both groups had greater levels than non-Hispanic Caucasians [56]. Likewise, in the South Carolina Cardiovascular Disease Prevention Program (SCCDPP), HbA_{1c} levels remained 0.3 and 0.4% significantly higher in African American non-diabetic men and women than in Caucasians [57].

Use of the HbA_{1c} \geq 6.5% (48 mmol/mol) cut point, to OGTT and FPG, was shown to detect different populations with T2D [58-60], resulting in both false positive and false negative diagnosis compared to glucose testing [61-63]. An assessment between the markers has shown that individuals with increased glycation of haemoglobin, i.e., high glycaters, would have higher HbA_{1c} levels whilst those with decreased glycation, i.e., low glycaters, would have lower HbA_{1c} levels than that expected from their FPG values [55]. Non-Hispanic African Americans have been reported to have higher HbA_{1c} at any given value of FPG or 2-hr glucose than Non-Hispanic Caucasians [64]. Again when ethnic differences were included,

for a given value of mean blood glucose, HbA_{1c} values have been reported to be 0.27%, 0.32% and 0.42% higher in Asians, Hispanics and Africans respectively [65]. Similar differences have also been observed in other multi-ethnic studies in the UK [66] and Singapore [67].

The importance of genetics on heritability of HbA_{1c} has been established in twins [68,69] and is associated with non-glycaemic determinants of HbA_{1c} [70] and in part has been shown to explain the greater and differential susceptibility of Africans and East Asian sub populations to developing T2D than Caucasians [71]. Hence the body of evidence suggests that a set universal cut off point of HbA_{1c} may not be applicable across all ethnic groups, and that ethnic specific cut offs may be needed to identify individuals at risk. This rationale was endorsed in a study conducted in Taiwanese individuals in whom the predicted time course for progression to T2D at HbA_{1c} 6.5% was found to be 2.49 years [72]. Was this study to be conducted in a multi-ethnic population the prediction may likely increase or decrease. Furthermore embedded within these complexities, is that age may also impact of the levels of HbA_{1c} with a recent study by Yan *et al* [73] demonstrating that optimal cut offs for HbA_{1c} for diagnosing T2D were 5.7% in young and middle aged group (39.9 \pm 8.0 years) and this increased to 5.9% in the elderly group (71.6 \pm 6.7 years) whilst for prediabetes thresholds were at 5.6% and 5.7%, respectively.

To circumvent some of these criticisms with the ADA HbA_{1c} threshold, the use of two cut points, one to “rule-out” (HbA_{1c} \leq 5.5%) and the second to “rule in” (HbA_{1c} \geq 7.0%) T2D has been recommended [74]. The lower value was chosen for its 95% negative predictive value to rule out T2D whilst the upper limit, although higher than the recommended 6.5% cut off, would optimise the specificity of the test.

Standardisation of Methods and Reporting Units

The clinical relevance of the test has also been underscored by lack of standardization of the HbA_{1c} assay in terms of methodology and Units as reported between countries [75]. Efforts to unify assay methodologies according to reference standards were initiated by the National Glycohaemoglobin Standardization Program (NGSP), the Diabetes Control and Complications Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) [76]. The International Federation of Clinical Chemistry (IFCC) also subsequently developed an accuracy based definitive reference system for HbA_{1c} that is now recognised to be a worldwide reference standard [77]. Although the NGSP/DCCT/UKPDS and IFCC systems are well correlated, the number scales differ with the latter reporting HbA_{1c} as concentration (mmol/mol) and the former as glycation percentage (%).

Assay methods may also impact measured HbA_{1c} and requires that reported levels be viewed with caution as they may not truly reflect glycaemic control over 2-3 months in the presence of haemoglobinopathies, i.e., haemoglobin variants such as HbS, HbC, HbE and HbF, which have the potential to interfere with the assay methods [78]. Red cell turnover too may impact results; falsely raised or conversely decreased HbA_{1c} concentrations are observed due to increased or decreased

circulating erythrocyte life span [79] or impaired reticulocyte production and haemolysis respectively. Moreover, assay measurements could also be affected by greater micronutrient intake such as Vitamin C and E which have the potential to reduce rates of glycation; however the degree to which this occurs at pharmacological doses is not determined [80]. Similarly iron deficiency anaemia would cause an overestimation [81] in measured HbA_{1c}. This has been recently reported in a study conducted in Asian Indians, wherein the prevalence of prediabetes and T2D using HbA_{1c} was 25.9% whilst using OGTT was only 10.4%, the dissimilarity due to one-third anaemic participants [82]. Similarly, inter individual differences in intra-erythrocyte 2,3-diphosphoglycerate that catalyses the production of HbA_{1c} may also account for variability in test results [83]. Female sex hormones [84] and differences in visceral fat [85] have also been shown to contribute to inconsistencies in measured values.

Adopting a Buddy System - Combining HbA_{1c} with 2-H OGTT or FPG to Improve Diagnostic Accuracy for Risk Prediction

A recent large meta-analysis based on studies that adopted the WHO and/or ADA criteria for prediabetes reported that isolated HbA_{1c} is neither sensitive nor specific for diagnosing the intermediate state of prediabetes [12]. The analysis confirmed FPG to be more likely to be specific, with respective ADA (2010) HbA_{1c} cut points of <6.5% and <5.7% not reliably excluding the presence of T2D and prediabetes. Differences in pathophysiological mechanisms, phenotype and other factors affecting HbA_{1c} could all explain the observed discordance between FPG/OGTT and HbA_{1c} classification of prediabetes [24,73,86,87]. The joint guidelines by the European Society for Cardiology (ESC) and the European Association for the Study of Diabetes (EASD) have recommended that an OGTT still be conducted in the absence of positive HbA_{1c} diagnosis in those individuals identified as at risk based on simple phenotypic characteristics and/or medical history including ethnicity, age, body mass index (BMI), hypertension and prior stroke [88]. Some researchers have recommended a shortened 1 h glucose measurement (glucose \geq 8.6 mmol/L) during OGTT be used with HbA_{1c} for diagnosis of prediabetes [89-92]. Others note that combined use of FPG and HbA_{1c} identifies greater numbers of T2D than HbA_{1c} alone (52.2% vs. 32.3%) [93], again with improved diagnostic sensitivity and specificity [73,94] for early identification of high risk prediabetics, with increased Hazards Ratio of 38.6 (95% CI 27.6–54.0) [24] than single markers alone. Since each marker for diagnosis has its own limitations it appears likely that including either OGTT and/or FPG with HbA_{1c} would improve sensitivity and specificity to predict at risk individuals [95] and so better represent different facets of metabolic progression through prediabetes to T2D [27].

Conclusion

Traditionally, the diagnosis of prediabetes and T2D has relied on the measurement of glucose concentrations in timed samples, such as FPG; in random samples independent of

prandial status and/or OGTT. The introduction of HbA_{1c} by ADA in 2010 as diagnostic criteria was based on the premise that it would afford cheaper, more convenient and accurate screening of high risk individuals. However subsequent use of the marker within the last decade has led several published works to suggest that its sensitivity and specificity to diagnose high risk cohorts needs to be readdressed. HbA_{1c} is limited by ethnic, racial and gender differences, possibly a result of differences in glycation and/or red cell survival, and clearly identifies a different pool of individuals as prediabetic compared to those identified by FPG or OGTT and cohort overlap can be low. Better understanding of the limitations of HbA_{1c} may improve prediction and diagnostic accuracy, much needed in order to identify those individuals who require fast track into lifestyle modification [100-102] and/or other therapeutic programs [103,104] currently, used as a complementary test to FPG or OGTT remains likely to be the approach that is most beneficial.

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