HbA_{1c}: how do we measure it and what does it mean?

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Purpose of review

Description of recent developments in the standardization of HbA_{1c} measurement and interpretation of HbA_{1c} results.

Recent findings

HbA_{1c} is extensively used in the management of patients with diabetes. The two major schemes to standardize HbA_{1c} produce values that differ substantially. A prospective, multinational study revealed a linear correlation between HbA_{1c} and average blood glucose. Some, but not all, assay methods are able to accurately measure HbA_{1c} in individuals with common hemoglobin variants.

Summary

Progress in standardization of methods for HbA_{1c} measurement has significantly reduced variation among different methods. The improved accuracy could allow HbA_{1c} to be used for screening and diagnosis of diabetes. A consensus document recommends that HbA_{1c} be reported in both NGSP (%) and IFCC (mmol/mol) units. HbA_{1c} results can be translated into estimated average glucose (eAG), which could be reported in addition to HbA_{1c}.

Keywords

diabetes mellitus, glycated hemoglobin, HbA_{1c}, hemoglobin variants, standardization

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Introduction

The global prevalence of diabetes mellitus is increasing rapidly. Diabetes currently affects 246 million people worldwide and is expected to affect 380 million by 2025 [1]. Measurement of glycated hemoglobin, predominantly HbA_{1c}, is fundamental to the management of patients with diabetes. HbA_{1c} is used to monitor long-term glycemic control, adjust therapy, assess the quality of diabetes care and predict the risk for the development of complications [2–4]. Accurate and reliable methods to measure HbA_{1c} are necessary for optimal use.

Glycation of hemoglobin

Glycated hemoglobin is derived from the nonenzymatic addition of glucose to amino groups of hemoglobin. HbA_{1c} is a specific glycated hemoglobin that results from the attachment of glucose to the N-terminal valine of the hemoglobin β-chain [5]. Total glycated hemoglobin includes all glycated fractions, comprising HbA_{1c} as well as hemoglobin glycated at sites other than the N-terminus of the beta chain e.g., epsilon amino groups on lysine residues. The concentration of HbA_{1c} depends on both the concentration of glucose in the blood and the life span of the erythrocyte. Because erythrocytes are in the circulation for approximately 120 days, HbA_{1c} represents the integrated glucose concentration over the preceding 8–12 weeks [2]. It is, therefore, free of the large fluctuations that occur daily in blood glucose concentrations.

Measurement of HbA_{1c}

The existence of several forms of hemoglobin has been known for over 50 years and 'an unusual hemoglobin' was described in patients with diabetes in 1969 [6]. Numerous assays were subsequently developed to measure glycated hemoglobins. The principle of all methods is to separate the glycated and nonglycated forms of hemoglobin. This can be accomplished based on differences in charge (usually by HPLC) or structure (usually immunoassays or boronate affinity chromatography). There was minimal assay standardization initially and results varied widely among methods [6]. Programs were developed in the 1990s in a few countries, most notably Sweden, Japan and the USA, to standardize HbA_{1c} measurements [6].

The most widely adopted system is that of the National Glycohemoglobin Standardization Program (NGSP), which standardizes glycated hemoglobin test results so that values reported by clinical laboratories are comparable to those reported in the two largest clinical trials on the effects of intensive diabetes treatment, namely the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) [7]. NGSP-certified methods are used worldwide. The NGSP standardization process has significantly reduced

the variation in glycated hemoglobin measurement among laboratories [7]. Despite the considerable improvement, intermethod variability is reported to still be a potential source of inaccuracy [8]. Efforts to further enhance the accuracy of HbA_{1c} measurements are being actively pursued [9].

The International Federation for Clinical Chemistry (IFCC) developed a reference method for measuring HbA_{1c} [10]. An *N*-terminal hexapeptide is cleaved from the β-chain of hemoglobin by the enzyme endoproteinase Glu-C. Glycated and nonglycated hexapeptides are separated from one another by high performance liquid chromatography and separately quantified by either mass spectrometry or capillary electrophoresis. The IFCC reference system produces values that are 1.5–2.0% absolute HbA_{1c} units lower than those measured by the NGSP [11], presumably due to the greater specificity of the IFCC method. A network of 13 reference laboratories has been established to form an IFCC reference system [12•]. The main function of the IFCC system is to serve as an 'anchor' for HbA_{1c} and allow manufacturers to calibrate their instruments to a higher level reference method. The IFCC method is time consuming, technically complex and carries higher cost, thus is not designed to be used for routine analysis of patient samples.

How is HbA_{1c} reported?

HbA_{1c} is usually reported as a percentage of total hemoglobin. The NGSP values, which are equivalent to those reported in the DCCT and UKPDS, have been used most widely. This enables a patient's results to be directly compared with those clinical outcomes studies. To avoid confusion with the widely used NGSP/DCCT/UKPDS units and to conform with Systeme International (SI) Units, IFCC numbers are now reported as mmol/mol [13•,14]. For example, an HbA_{1c} result of 7% (in NGSP/DCCT/UKPDS units) is equivalent to 53 mmol/mol (in IFCC units). A consensus paper on worldwide standardization of HbA_{1c} [15•] recommends that HbA_{1c} values be reported in IFCC (mmol/mol) and NGSP (%) units, as well as average glucose. Although a uniform system for reporting HbA_{1c} is desirable, it is likely that different formats will be adopted in different countries.

As mentioned earlier, HbA_{1c} reflects blood glucose concentrations over the preceding 8–12 weeks and is commonly used as an indication of average blood glucose concentration. Retrospective analysis of data in the DCCT indicates a linear correlation between HbA_{1c} and average glucose [16]. However, the DCCT was limited to patients with type 1 diabetes and was not designed to measure average glucose. A multinational study (termed A1c Derived Average Glucose or ADAG) was recently performed to ascertain the relationship between HbA_{1c} concentrations and long-term glucose values [17••]. The 507 study participants comprised patients with type 1 or type 2 diabetes as well as nondiabetic individuals. Broad ethnic and racial representation was obtained by recruiting individuals at 10 centers in the USA, Europe, Africa and Asia. To evaluate average glucose, participants performed a combination of continuous glucose monitoring and regular self-monitoring of blood glucose using portable meters. Over the course of the 12-week study, each participant had approximately 2700 glucose measurements. Comparison of HbA_{1c} and average glucose results reveals a linear correlation [$AG_{mg/dl} = 28.7 \times Hb\ A_{1c} - 46.7$ ($AG_{mmol/l} = 1.59 \times Hb\ A_{1c} - 2.59$)] [17••]. For example, an HbA_{1c} value of 6% (42 mmol/mol) (equivalent to the upper limit of the reference interval) translates into average glucose of 126 mg/dl (7.0 mmol/l). The regression equation produces values approximately 11% lower than those obtained from the DCCT, perhaps because average glucose was measured more accurately in the ADAG study. A smaller study (22 participants) that included continuous glucose monitoring for 3 months derived a relationship similar to that in the ADAG study [18].

The ADAG study has some limitations. For example, the average glucose varies among individuals with the same HbA_{1c} concentration. Several factors could account for the scatter. These include measurement error, interindividual variation, differences in glycation or differences in red cell turnover rates. In addition, the study enrolled only diabetic patients with stable glycemic control, few Asians and no children or pregnant women. Notwithstanding these limitations, the regression equation can be used to calculate an eAG (estimated average glucose) based on the HbA_{1c} result. This eAG value would not replace the measured HbA_{1c} concentration, which would still be reported, but could be provided in addition to the HbA_{1c}. The eAG, reported in familiar glucose units (i.e., mg/dl or mmol/l), could be used to help patients understand the meaning of HbA_{1c} and how to use it appropriately to improve their glycemic control. This postulate is supported by the demonstration that improving patients' knowledge of the relationship between HbA_{1c} and average glucose improves glycemic control [19]. However, the concept of expressing HbA_{1c} in terms of average glucose is not accepted by all and remains controversial [20–22].

Limitations of HbA_{1c} testing

For the vast majority of patients with diabetes, HbA_{1c} provides an excellent measure of glycemic control. However, there are situations where HbA_{1c} may be unreliable. These include any condition that alters the erythrocyte life span (e.g., hemolytic anemia), severe iron-deficiency

anemia, and certain hemoglobin variants or adducts, or recent red blood cell transfusions. Factors such as race or age are also reported to influence HbA_{1c}.

HbA_{1c} variability

The intraindividual variation of HbA_{1c} in nondiabetic individuals is very low (<2%) [23,24], but substantial interindividual (between individuals) variation occurs. Moreover, there are published reports of diabetic individuals who appear to have HbA_{1c} values that are higher or lower than expected based on their clinical presentation, blood glucose results, glycated plasma proteins (e.g., fructosamine), or home glucose monitoring data [25•,26]. Since obtaining accurate mean blood glucose (MBG) is problematic, it has been difficult to determine the cause(s) of these discrepancies and to verify that these differences are independent of MBG. This disparity between HbA_{1c} and other measures of glycemia, termed the 'glycation gap' or 'hemoglobin glycation index', has a genetic component [25•]. Some authors have proposed a theory of high and low glycators [25•,26], whereby individuals with the same MBG may have different HbA_{1c} concentrations. However, there is currently no reliable way to directly measure glycation rates *in vivo* and the hypothesis of different glycation rates is not substantiated by data.

Differences in erythrocyte life span might account for some of these disparities. Although the 'average' erythrocyte life span is 120 days, there is a range of values among individuals. For example, a recent study in a very small group of individuals ($n = 12$) showed that erythrocyte survival varies sufficiently among 'hematologically normal' people to cause clinically important differences in HbA_{1c} [27]; this would infer variability in HbA_{1c} that is not related to glycemic control. Notwithstanding these observations, long-term clinical outcomes studies have clearly demonstrated very strong correlations between HbA_{1c} concentrations and risks for complications in patients with diabetes [28,29]. Moreover, HbA_{1c} predicts risk of cardiovascular disease even within the 'normal' HbA_{1c} range [30]. Therefore, although parameters independent of glycemia may influence the variability of HbA_{1c}, these appear to be much less clinically significant than the impact of glycation on diabetes complications.

Race might influence HbA_{1c}. Statistically significant differences in HbA_{1c} concentrations among races have been reported in those with diabetes [31–33], even after adjustment for covariates such as quality of care. For example, in the TRIAD study, Latinos, Asians, and African–Americans had absolute HbA_{1c} values 0.4, 0.4, and 0.2%, respectively, higher than whites [32]. Although these studies adjusted for factors likely to affect glycemia, one cannot exclude the possibility that the differences among these populations may be due to differences in glycemic control. Herman *et al.* [34•] analyzed a cohort of adults with impaired glucose tolerance and identified differences in HbA_{1c} among different racial groups. Racial differences in HbA_{1c} have also been observed in nondiabetic populations [35,36], which suggest that there are heritable variations in HbA_{1c}. The underlying mechanism is not known. Possibilities include differences in rates of glucose entry into erythrocytes, rates of glucose attachment to or release from hemoglobin, or erythrocyte survival. Regardless of the cause, the variations in HbA_{1c} found among racial/ethnic groups are relatively small ($\leq$0.4% HbA_{1c}) and may not be clinically significant.

HbA_{1c} increases with age by approximately 0.03% per year in nondiabetic individuals [37•,38]. Some conclude that the increase in HbA_{1c} independent of the well documented decline in glucose tolerance with age is minimal [39]. The small increase is unlikely to necessitate a change in treatment goals for different age groups.

Factors that interfere with the measurement of HbA_{1c}

Hemoglobin variants affect some HbA_{1c} measurements. The most common variants worldwide (in descending order of prevalence) are HbS, HbE, HbC and HbD. (In the USA, HbC is more common than HbE.) In addition, HbF may be increased in some conditions (e.g., leukemia, anemia) or hereditary persistence of fetal hemoglobin [40]. No HbA_{1c} method is appropriate for assessment of glycemic control in patients homozygous for HbS or HbC, with HbSC disease, or with any other condition that alters erythrocyte survival. Generally, individuals heterozygous for hemoglobin variants do not have shortened erythrocyte survival and HbA_{1c} can be measured accurately if an appropriate assay method is used. Several publications have analyzed the effects of these hemoglobins on HbA_{1c} results [41•–43•] (reviewed in [44]). The published findings are summarized in Table 1 and on the NGSP website (www.ngsp.org). The interferences are usually method specific. In general, HbAS and HbAC interfere with some immunoassays, whereas HbAE and HbAD interfere with some HLPC methods (Table 1). If an HPLC method is used, careful inspection of chromatograms usually reveals aberrant peaks produced by the variants, enabling detection of unacceptable results. As with any test, results that contradict the clinical picture should be investigated further.

Factors that affect the interpretation of HbA_{1c} results

Iron deficiency anemia, a major public health problem in developing countries, is associated with higher HbA_{1c} and higher fructosamine concentrations [40]. Consistent

Table 1 Interference of heterozygous variants S, C, D, E and increased HbF with specific HbA_{1c} methods

		Interference from				
Manufacturer	Method	HbAS	HbAC	HbAE	HbAD	HbF
Immunoassay						
Abbott	Architect/Aeroset	Yes	Yes	–	–	–
Bayer (Metrika)	A1cNOW	Yes	Yes	No	No	–
Beckman	Synchron System	No	No	No	No	–
Dade	Dimension	No	No	No	No	–
Olympus	AU system	Yes	Yes	No	No	–
Orthoclinical	Vitros	No	No	No	No	–
Point scientific	HbA1c on Modular P	No	No	No	No	–
Roche	Cobas Integra[c]	Yes	Yes	–	–	–
Roche	Cobas Integra Gen.2 (Tina Quant)	No	No	No	No	–
Roche/Hitachi	Hitachi (Tina Quant)	No	No	No	No	–
Siemens (Bayer)	Advia	Yes	Yes	–	–	–
Siemens (Bayer)	DCA 2000	No	No	No	No	Yes[a]
Ion-Exchange HPLC						
Bio-Rad	D-10 (short)	No	No	No	No	–
Bio-Rad	D-10 (extended)	No	No	No	No	–
Bio-Rad	Variant A1c	No	No	No	Yes	–
Bio-Rad	Variant II A1c	No	No	No	No	No
Bio-Rad	Variant II Turbo A1c	No	No	Yes	Yes	–
Menarini	HA8140 (diabetes mode)	Yes	No	–	–	–
Menarini	HA8160 (diabetes mode)	No	No	Yes	Yes	–
Menarini	HA8160 (TP mode)	No	No	No	Not Quantified	–
Tosoh	A1c 2.2 Plus	No	No	Yes	No	Yes[a]
Tosoh	G7	No	No	Yes	No	No[b]
Tosoh	G8	–	–	Yes	No	–
Boronate affinity						
Axis-shield	Afinion	No	No	No	No	–
Primus	Boronate affinity HPLC	No	No	No	No	Yes[a]
Other						
Diazyme	Direct enzymatic A1c	No	No	No	No	–

HPLC, high performance liquid chromatography.
[a] HbF concentrations above 15% cause clinically significant low bias.
[b] Offline manual recalculation must be performed if the HbF peak is mislabeled as labile HbA_{1c}.
[c] Method being phased out.

with these observations, iron replacement therapy lowers both HbA_{1c} and fructosamine concentrations in diabetic and nondiabetic individuals [40,45,46]. Similarly, HbA_{1c}, but not glycated albumin, is increased in late pregnancy in nondiabetic individuals owing to iron deficiency [47•]. Insight into the mechanism was recently obtained by the observation that malondialdehyde, which is increased in patients with iron deficiency anemia [40], enhances the glycation of hemoglobin [48]. Alternative measures of glycemic assessment (e.g., glucose monitoring) must be used in the presence of significant iron deficiency anemia, at least until the iron deficiency has been successfully treated.

Chronic renal failure develops in many diabetic patients. Almost half of all individuals with end stage renal disease in the USA have diabetes [49]. The role of glycemic control and the value of HbA_{1c} in diabetic individuals with renal disease are controversial. Although some studies detect no correlation between HbA_{1c} and survival in dialysis patients [50,51], others observe that higher HbA_{1c} is incrementally associated with increased risk of death in diabetic patients undergoing maintenance hemodialysis [52]. Lower HbA_{1c} is associated with improved survival in these patients, provided the decreased HbA_{1c} does not result from malnutrition or anemia [52], suggesting that better glycemic control is important for this population. A recent report suggests HbA_{1c} underestimates glycemic control in diabetic patients on dialysis and that glycated albumin is a more robust indicator of glycemic control [53•]. Further studies are needed to clarify the role of HbA_{1c} in diabetic patients with chronic renal failure.

HbA_{1c} for screening and diagnosis of diabetes

It is estimated that 25% of people with diabetes in the USA have not been diagnosed [54]. Moreover, at the time of diagnosis, 25% of patients have diabetic retinopathy or microalbuminuria [55]. Earlier diagnosis of diabetes could prevent or delay these complications.

The use of HbA_{1c} for screening and diagnosis of diabetes has been debated extensively for over 25 years [16,56–58]. Advantages and disadvantages for HbA_{1c} are listed in Table 2. A review of the literature (published in 2007) concluded that HbA_{1c} is as effective a screen as fasting plasma glucose for the detection of type 2 diabetes [59••]. A committee of experts recently recommended that

Table 2 HbA_{1c} for screening and/or diagnosis of diabetes*

Advantages	Disadvantages
The patient need not be fasting	Limited studies
HbA_{1c} is highly correlated with diabetes complications	Other conditions may alter HbA_{1c} values (e.g., variant Hb, uremia, transfusion)
HbA_{1c} is widely used as a measure of glycemic control in patients with diabetes	Question whether precision and accuracy adequate
Measurement of HbA_{1c} is now standardized and the accuracy of the test is monitored	High cost; more expensive than glucose
The current recommended diagnostic criteria are often not followed in the community setting	Limited availability in some areas of world
Many physicians already use HbA_{1c} for screening and diagnosis	Cut-off not established
Intra-individual variability of HbA_{1c} (<2%) is considerably lower than that of fasting plasma glucose	
Indicates chronic hyperglycemia and is not affected by short-term lifestyle changes	
A single test for diagnosis and monitoring is attractive	
Threshold value associated with risk for retinopathy, similar to that for glucose	

* Except in Japan, HbA_{1c} is not currently recommended for screening or diagnosis of diabetes.

HbA_{1c} be incorporated into criteria for screening and diagnosis of diabetes [60••]. The panel suggested that HbA_{1c}, at least 6.5%, would be diagnostic of diabetes if confirmed by an increased blood glucose value. Moreover, the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) have established a joint committee to reevaluate the diagnosis of diabetes; HbA_{1c} is under consideration (David Nathan, personal communication). Appropriate cutoffs would have to be established and it is possible that thresholds for screening and diagnosis could differ [60••]. Nevertheless, it seems likely that HbA_{1c} will soon be recommended as a screening/diagnostic test for diabetes.

Conclusion

HbA_{1c} measurement is integral to the management of individuals with diabetes. Both the variability among methods that measure HbA_{1c} and the interference produced by variant hemoglobins have been significantly reduced. Ongoing efforts are being directed towards further improving the accuracy of HbA_{1c} measurement. This progress may enable HbA_{1c} to be used for screening and diagnosis of diabetes.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 192–193).

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