Chapter 37

Hemoglobin A1c: Consensus and Controversy

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INTRODUCTION

In diabetes mellitus, the therapeutic goal is to maintain the blood sugar levels as close to normal as possible and to prevent/delay complications. This requires close monitoring of the glycemic status. Assessment of the glycemic status can be done by measuring the fasting plasma glucose (FPG), postprandial glucose (PPG), Hemoglobin A1c (HbA1c) or by continuous glucose monitoring systems (CGMS).

However, glucose measurement itself is less accurate than most clinicians realize. Recent analysis showed that greater than 12% of classification errors of diabetes occurred due to technical problems in assays of plasma glucose. Pre-analytic errors, like sample collected in fluoride bulb but kept at room temperature for 1–4 hours, can decrease plasma glucose level by 3–10 mg% in nondiabetics. Moreover, 8 hours of fasting before FPG may not be rigidly followed by all subjects.1

Oral glucose tolerance test (OGTT) is a cumbersome procedure and hardly feasible in routine clinical practice.

WHAT IS HbA1c?

Different types of glycation products are formed from the HbA1c chain depending upon the carbohydrate moiety. When glucose is added to the N-terminal valine of the hemoglobin beta chain, it forms a nonenzymatic glycated product, HbA1c, which is less than 6% of the total hemoglobin. The number 1c following Hba represents the order in which this hemoglobin is detected on chromatography.

Even though, HbA1c is said to reflect the past 3 months of glycemic status, glycation of hemoglobin continues throughout the entire period, with recent glycemic status having the greatest influence on the HbA1c values. Mean blood glucose of the last 1, 2 and 3 months contribute about 50%, 40% and 10% respectively to the final A1c value.

The Diabetes Control and Complications Trial (DCCT) demonstrated a direct correlation between glycemic control as indicated by A1c, and the likelihood of developing long-term diabetes-related complications.2 In the general population, FPG is a poor marker of future cardiovascular events, whereas 2-hour OGTT and A1c are good predictors.3

In an individual, the correlation is stronger among two A1c measurements than among the FPG or 2-hour PPG measurements. The coefficients of variation of A1c, FPG and 2-hour PPG are 3.6, 5.7 and 16.6%, respectively,4 reflecting both biological and analytical variability. However, although the latter was similar for A1c and FPG (~2%), biological variability of A1c is several fold lower than that of FPG (~1 vs ~4%).5 Hence, two required assessments of FPG to diagnose diabetes can provide quite unreliable information, whereas A1c, especially if measured twice as recommended, provides more robust clinical information.

Factors affecting HbA1c measurements are acute hyperglycemia, severe anemia, gestation, red blood cell life span, abnormal hemoglobin, hypertriglyceridemia, etc.

Methods of Estimation

- **High performance liquid chromatography:** It is considered as the gold standard method; ion exchange high performance liquid chromatography (HPLC) method is based on the charge of the globin component of hemoglobin and although it measures all types of hemoglobin and is affected by abnormal and minor hemoglobin fractions, the DCCT and the National Glycohemoglobin Standardization Program (NGSP) have recommended it as an acceptable standard.

- **Immunoturbidimetric method:** Here HbA1c antibody used in turbidimetric immunoassays reacts only with HbA1c and the result can be measured easily with a turbidimeter. These methods are easier to adapt to biochemical devices, cheaper in cost and faster in producing results than HPLC, but despite all these advantages, turbidimetric immunoassays have lower precision than HPLC.

- **Affinity chromatography**

- **Electrophoretic methods**

- **Methods based on chemical reactions**

In view of the various methods of estimation available, the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), the International Diabetes Federation (IDF), the International Federation of Clinical Chemistry (IFCC) and the International Society for Pediatric and Adolescent Diabetes recently published an updated consensus statement regarding global standardization of HbA1c, the original having been issued in 2007.6 The main recommendation was that the HbA1c results are to be reported by clinical laboratories worldwide in SI (Systeme International) units (mmol/mol)—no decimals) and derived NGSP units (%—one decimal), using the IFCC-NGSP master equation (DCCT units), i.e. as HbA1c value (NGSP) in %, HbA1c value (IFCC) in mmol/mol and estimated average glucose (eAG) in mg/dL. A typical standard report is shown in Figure 1.

High HbA1c levels may be seen in newly diagnosed diabetes, uncontrolled diabetes, noninsulin dependent hyperglycemia—acromegaly, pheochromocytoma, thyrotoxicosis, Cushing’s syndrome, splenectomy, alcoholism, iron deficiency and other states of decreased red cell turnover.
Low HbA₁c levels maybe seen in hemolytic anemias—congenital (spherocytosis/elliptocytosis) and acquired (drug induced—dapsone/methyldopa), hemoglobinopathies, chronic blood loss, chronic renal failure (especially if on erythropoietin).

HbA₁c AS A DIAGNOSTIC CRITERION

Traditionally, diagnosis of diabetes is made with FPG and/or 75 gm OGTT results: FPG greater than 126 mg%; 2-hour plasma glucose greater than 200 mg%. However, more recently, A₁c greater than 6.5% has also been recognized as a diagnostic criterion (to be confirmed with a repeat A₁c test). The ADA standards of care specifically mentioned that the diagnostic test should be performed using a method certified by the NGSP or traceable to the DCCT reference assay.

However, this criteria has created a lot of controversy as inconsistencies in the correlations between glycated hemoglobin and other measures of glycaemia have been reported in different ethnic and racial groups, suggesting genetic influences on hemoglobin glycation. For example, blacks appear to have slightly higher A₁c (an absolute increase of 0.2–0.3%) than whites.

Yusuke Tsugawa showed that the prevalence of retinopathy increased at lower HbA₁c levels for black participants than for white participants (5.5–5.9% vs > 6.5%).

Lee et al. (EASD 2010) found that the cut-off value of HbA₁c for diagnosing diabetes varied for different ethnic groups—6.0% with a sensitivity and specificity of 88.2% and 79.9% respectively, as compared to ADA proposed value of 6.5%. Moreover, adjustment of this value is needed with age: 6.0% at ages 21–40 years, 6.2% at ages 41–60 years and 6.4% at ages greater than 60 years.

In another study, which included data from CURES from India, Christensen DL et al. showed a significant impact on the prevalence figures of diabetes in different ethnic groups, when using A₁c as a diagnostic criterion as compared to OGTT.

The molecular mechanism underlying the racial and ethnic differences remains to be established. Regardless of the mechanism, the variations in A₁c consensus are relatively small (< 0.4%), and no consensus has been reached on whether different cut-offs should be used for different races. Longitudinal studies with larger samples are needed to determine lower cut-offs of HbA₁c levels for diagnosis in blacks.

Even though the cut-off of HbA₁c level for diagnosis of diabetes is 6.5%, studies have shown that HbA₁c even in the range of 5.5–6.5% poses considerably high risk of morbidity and mortality due to cardiovascular disease. Each 1% increase in HbA₁c poses 15% and 18% relative risk of cardiovascular disease in Type I diabetes mellitus and Type II diabetes mellitus respectively. Moreover, prospective studies indicate that a high normal HbA₁c level of 5.5–6.5% poses very high risk for subsequent development of diabetes, and the risk increases substantially as the values increase.

Clinical Implications of Using A₁c as a Diagnostic Criteria

- Offers greater convenience and accuracy than glucose measurements and correlates well with long-term complications
- May be too expensive for routine use in some parts of world
- May be influenced by hemoglobin traits and precluded for people with conditions that affect red cell turnover as in hemolytic anemia and chronic malaria
- HbA₁c is not the “gold standard” for diabetes diagnosis as no single assay can define the relationship between glucose and vascular complications
- The American Diabetes Association endorses committee recommendations on HbA₁c “in principle” while evaluating implications.

HbA₁c FOR MONITORING OF GLYCEMIC CONTROL

For monitoring, normal goals are less than 6.5% (American Association of Clinical Endocrinologists); less than 6.5% (IDF) and less than 7% (ADA). HbA₁c measures mean glycemic exposure during the preceding 2–3 months, and does not provide information about day-to-day changes in glucose levels. Being an integrated measure of overall glucose exposure, patients with Type II diabetes mellitus with similar A₁c values may have very different glucose profiles. Studies have shown a strong correlation between mean plasma glucose (MPG) and HbA₁c. MPG is the average of a multiple of measurements of glucose throughout the day. With each 1% change in HbA₁c, there is a change in about 35 mg/dL MPG.

Hanefeld et al. depicted postprandial hyperglycemia as a better predictor of subsequent myocardial infarction and cardiovascular mortality than fasting hyperglycemia. Further landmark studies have confirmed this finding suggesting that postprandial hyperglycemia is an independent risk factor for macrovascular disease.

Rosediani et al. (Malaysia) showed that PPG had a better correlation with HbA₁c than FPG (r = 0.604 vs 0.575).
**Diabetology**

In another prospective intervention trial to assess the relative contribution of FPG and PPG in achieving recommended HbA1c goals in 164 patients, it was seen that only 64% of patients achieving FPG targets of less than 100 mg/dL achieved a HbA1c target of less than 7%, whereas this target was achieved in 94% of patients maintaining the postprandial target of less than 140 mg/dL. PPG accounted approximately for 80% of HbA1c when HbA1c was less than 6.2% and only about 40% when HbA1c was above 9.0%.26

Recently, the Insulin Resistance Atherosclerosis Study (IRAS) showed that HbA1c is a weaker correlate of insulin resistance and secretion as compared with FPG and 2-hour PPG.24

In the KORA F4 study, it was shown that there was a J-shaped association between clinical distal sensorimotor polyneuropathy and quartiles of 2-hour postchallenge glucose, but not with FPG or HbA1c levels.25

Hence, it is important to realize that for ideal glycemic monitoring, besides HbA1c, other issues like PPG and glycemic variability have to be taken into account.

**PROS AND CONS OF HbA1c**

**Advantages of HbA1c**

- Fasting status not required for measurement
- Low biologic variability
- Marker of long-term glycemia
- Stable during acute illness
- Blood samples remain stable in vial, so that pre-analytical variability of A1c is negligible (unlike pre-analytical variability of 5–10% for FPG)
- Close association of results with complications
- Gives additional information based on individual susceptibility to glycation. It has been found that subjects with high hemoglobin glycation index (HGI) (defined as the difference between observed and predicted A1c level) had a greater risk of developing retinopathy and nephropathy despite good glucose control, and that subjects with lower HGI had a low incidence of microangiopathy despite high mean blood glucose levels, thus demonstrating that A1c assessment might provide not only information on chronic hyperglycemia but also a measure of whole body susceptibility of protein glycation and, therefore, risks of diabetes complications that are more strictly related to this pathogenic mechanism.

**Disadvantages of HbA1c**

- Lack of reliability in patients with hemoglobinopathies
- Unreliability in certain anemias with high red-cell turn over (e.g. hemolytic anemia, where HbA1c is usually reduced) or with low red-cell turn over (e.g. iron deficiency, where HbA1c is usually increased)
- Lack of reliability after recent transfusion (within previous 2-3 months)
- False low results in advanced renal disease
- Racial and ethnic differences in HbA1c levels
- Possibility of a glycation gap (differential glycation in response to the same ambient glucose exposure between persons)
- Captures only chronic hyperglycemia and will miss acute hyperglycemia; therefore, misses the importance of postprandial hyperglycemia
- Higher cost
- Lack of global availability and standardization.26

**INDIAN SCENARIO**

With very few accredited laboratories and limited resources, use of HbA1c as a diagnostic criterion, in India, is questionable. Even though, HbA1c provides several advantages, measure-ments of glucose are familiar and cheaper.

In an unorganized health sector, from rudimentary primary health centers to high-tech corporate hospitals, it is difficult to standardize a test like HbA1c all over the country, even for monitoring glycemic control. Till A1c is globally available in a standardized manner and the cost brought down, and further epidemiological and clinical data for ethnic and racial cut-offs are available, it is prudent to use FPG for diagnostic purposes, and PPG and FPG for monitoring. However, where adequate facilities are available, both A1c and FPG can coexist as diagnostic tools.27

**REFERENCES**

Section 5

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