Review

A Review: Assessment of Hba1c as A Diagnostic Tool in Diabetes Mellitus

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Abstract: **Background:** Hemoglobin A1C (HbA1c) measures the average plasma glucose level over the two to three months before by expressing the ratio of total to glycated hemoglobin. It has emerged as the most significant indicator of long-term hyperglycemia and is highly associated with late complications from diabetes. Now that the analysis has been standardized, it may be linked to international reference sources and reference techniques. The major method for identifying diabetes was made possible by the standardization of HbA1c analyses. The addition of HbA1c as a diagnostic tool will simplify the diagnosis of diabetes compared to the glucose criteria, and should increase the avoidance of late consequences of diabetes, despite some limitations in which HbA1c is not reflective of average glucose concentration.

Keywords: HbA1c; Diabetes mellitus; eAG; Hyperglycemia.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by rise in blood glucose level called hyperglycemia[1]. The worldwide prevalence of diabetes in 2000 was approximately 2.8% and is estimated to grow to 4.4% by 2030. This translates to a projected rise of diabetes from 171 million in 2000 to well over 350 million in 2030[2]. Diabetes is of two types, type 1 accounting for 5% prevalence and type 2 for 95% prevalence among diabetics. This calls for improved treatment of hyperglycemia and other risk factors associated with metabolic syndrome. Since it is possible to dramatically lower the risk of both micro and macrovascular complications [3]. Persistent elevations in blood sugar increase the risk for the long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, erectile dysfunction, neuropathy (loss of sensation, especially in the feet), gangrene and gastroparesis (slowed emptying of the stomach). Poor blood glucose control also increases the risk of short-term complications of surgery such as poor wound healing[2]. Glycated hemoglobin (glycosylated hemoglobin, hemoglobin A1C, HbA1c, A1C, or Hb1c; sometimes also HbA1c) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose[4]. The measurement of glycosylated hemoglobin (GHb) is one of the well-established means of monitoring glycemic control in patients with diabetes mellitus[5]. The use of hemoglobin A1C for monitoring the degree of control of glucose metabolism in diabetic patients was first proposed in 1976[6].

The clinical significance of HbA1c test was cemented by Diabetes Control and Complications Trial (DCCT) in type 1 diabetes 4 and the United Kingdom Prospective Diabetes Study (UKPDS) in type 2 diabetes[7]. The studies showed that HbA1c is an important marker in assessing a patient's risk of microvascular complications

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and hypoglycemia. Hence, measurement of both HbA1c and blood glucose levels are now used in the routine management of patients with type 1 and type 2 diabetes[8]. Measurement of glycated hemoglobin is recommended for both (a) checking blood sugar control in people who might be pre-diabetic and (b) monitoring blood sugar control in patients with more elevated levels. According to the American Diabetes Association guidelines the glycosylated hemoglobin test can be performed at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals[8].

2. Discussion

HbA1c is modified hemoglobin, with glucose linked to the N-terminal valine of the beta chain[9]. It is made in vivo by the non-enzymatic attachment of glucose to hemoglobin[10]. This occurs first by the formation of a labile adduct aldimine, which is a Schiff base that then rearranges to form a more stable keto-amine (Figure 1)[11]. The rate at which this reaction occurs is related to the prevailing glucose concentration, and it is expressed as a percentage of the total hemoglobin[12].

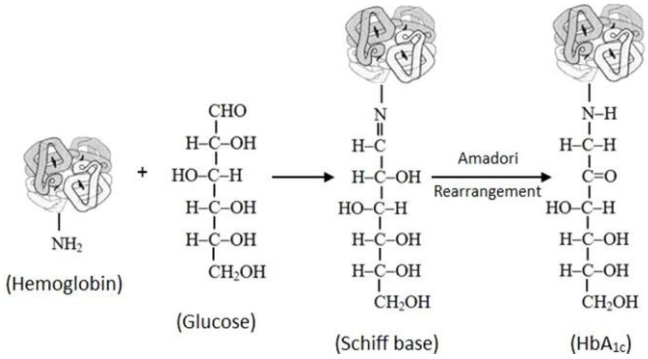

Figure 1 Formation of glycated hemoglobin (HbA1c) from the binding of glucose to hemoglobin.

2.1 Correlation Between HbA1c and Plasma Glucose

The relationship between A1c and Plasma glucose (PG) is complex. Higher levels of HbA1c are found in people with persistently elevated blood sugar, as in diabetes mellitus. A diabetic person with good glucose control has an HbA1c level that is close to or within the reference range. The International Diabetes Federation and American College of Endocrinology (IDFACE) recommend HbA1c values below 6.5%, while the American Diabetes Association (ADA) recommends that the HbA1c be below 7.0% for most patients[13]. On average, HbA1c of 6% corresponds to a mean plasma glucose of 135 mg/dL. For every increase in A1C of 1%, mean plasma glucose increases by 35 mg/dL. A normal non-diabetic HbA1C is 3.5-5.5%. In diabetes about 6.5% is good. Many studies have shown that A1C is an index of mean plasma glucose over the preceding weeks to months. A1C truly does not reflect glycemic control over last three months as it is claimed. Rather, it is weighted to the more recent weeks. The mean glycaemia during the month preceding the A1C measurement contributes 50% of the result, during the 30–60 days prior to the A1C measurements contributes another 25% and during the 60,120 days prior to the measurement contributes the final 25%[14]. HbA1c values corresponding to glucose level is given in Table 1. The approximate mapping between HbA1c values and *eAG* (estimated Average Glucose) measurements is given by the following equation[15].

$$eAG\ (\text{mg/dL}) = 28.7 \times \text{A1C} - 46.7 \quad \text{(Eq.1)}$$

$$eAG\ (\text{mmol/L}) = 1.59 \times \text{A1C} - 2.59 \quad \text{(Eq.2)}$$

A borderline (5.6–6.4%) or high (≥ 6.5%) level of HbA1c was found to strongly predict future drug treatment for diabetes mellitus[16].

Table 1 HbA1c values corresponding to glucose level[11].

HbA1c	*eAG* (Estimated Average Glucose)	
%	(mmol/L)	(mg/dL)
5	5.4 (4.2–6.7)	97 (76–120)
6	7.0 (5.5–8.5)	126 (100–152)
7	8.6 (6.8–10.3)	154 (123–185)
8	10.2 (8.1–12.1)	183 (147–217)
9	11.8 (9.4–13.9)	212 (170–249)
10	13.4 (10.7–15.7)	240 (193–282)
11	14.9 (12.0–17.5)	269 (217–314)
12	16.5 (13.3–19.3)	298 (240–347)

Data in parentheses are 95% confidence intervals.

2.2. Quantification of HbA1c

In measurement of HbA1c the prevalence of the most common hemoglobin variants (HbS, HbC, and HbD) depends on the genetic background of the population being analyzed. Single base pair mutations in the globin genes of hemoglobin, resulting in an amino acid substitution. More than 700 Hb variants are known and about half of these variants are clinically silent variant[17]. The presence of these Hb variants may falsely interfere with measurement of HbA1c by HPLC[18]. Hence the identification of Hb variants is important to avoid inaccurate Hba1c results. The degree of interference of Hb variants may vary with each method and even with each method modification[2].

The “true” HbA1c results may be obtained after appropriate correction based on the peak area for each glycated and no glycated component separated in the chromatograms. The HPLC method used by Camargo HPLC (Merck-Hitachi L-9100 Glycated Hemoglobin Analyzer; Tokyo, Japan) using a CCMpack Hb-S column in high-speed mode. This cation exchange column allows separation of Hb variants and the calculation for the glycated component is only related to HbA1c, not to HbS1c, HbC1c or to HbD1c resulting in very low GHb value. Hence it is recommended that laboratories measure GHb by a method that is not affected by Hb variants, rather than estimate it[19]. The development of automated HPLC method modification with high resolution mode aids the identification of interference caused by clinically silent hemoglobin variants in glycated hemoglobin (HbA1c) determination. Most HPLC systems are not able to resolve additional peaks in their chromatograms and this leads to the overestimation and underestimation of HbA1c results[20]. Affinity chromatographic methods measure glycol hemoglobin regardless of the glycation site and result in more accurate measure of glycemic control in samples with hemoglobin variants produced by hemoglobin mutations[21].

New methods are being developed, such as electrospray mass spectrometry[22] and a method based on quenching of the fluorescence of an eosin-boronic acid solution[23]. The immune agglutination method used was the DCA 2000 (Bayer, Vienna, Austria), which uses a specific antibody against the first six amino acid residues of the glycated N terminal of hemoglobin[20]. It was determined that only a few Hb variants are known to interfere with HbA1c results in immunoassays[24].

2.3. HbA1c in Monitoring Diabetes Control

The potential utility of HbA1c in management of diabetes was proposed in 1976 by Ronald Koenig and Anthony Cerami[25]; and a 1985 World Health Organization (WHO) report recommended its use for practical assessment of long term glycemic control in patients with diabetes mellitus. In individuals with a normal red blood cell lifespan, the level of HbA1c is related to the circulating plasma levels of glucose[26], as well as the average glycaemia of the previous 12 to 16 weeks, as this is the half-life of red blood cells[11]. HbA1c is

considered the gold-standard biochemical indicator of long-term glycemic control in diabetic patients[27], as a good association has been demonstrated with the chronic microvascular complications of diabetes, such as retinopathy, nephropathy and neuropathy[28]. HbA1c of less than 6.0% is considered normal, 6.0% to 7.5% is good control of diabetes mellitus, 7.6% to 9.0% is considered unsatisfactory control, and HbA1c of more than 9.0% is regarded as very poor control of diabetes mellitus. In Blantyre, Malawi, Cohen et al., in a survey of the management, control, and complications of diabetes mellitus in patients attending a diabetes clinic, found that 74% of patients had unsatisfactory levels of HbA1c (greater than 7.5%), and this was accompanied by a high frequency of microvascular complications[29].

2.4. HbA1c and Diabetes Diagnosis

The diagnosis of diabetes has traditionally been based upon the detection of elevated plasma glucose levels, either after fasting, two hours after an oral glucose (75 g) tolerance test (OGTT), or, in symptomatic individuals, after a random blood glucose check[30]. Recently, the American Diabetes Association and the WHO have recommended using HbA1c (greater than or equal to 6.5%) to diagnose diabetes mellitus[31]. This was based, in part, on the ability for HbA1c to predict clinical outcomes. It has been established that HbA1c has a similar relationship with prevalent diabetic retinopathy as that of both fasting and two-hour plasma glucose levels, and that lowering HbAlc can reduce microvascular complications[32,33]. Although HbA1c testing is currently more expensive than blood glucose measurements (the net cost of an HbA1c test is, on average, 13.6 times higher than a plasma glucose measurement), it provides significant practical advantages. HbA1c testing can be performed at any time of the day and does not require any special pre-test preparation by the patient (for example, overnight fasting or glucose loading)[34,35]. While the use of HbA1c for long-term glycemic control is well accepted, there remains significant controversy on its use as a diagnostic tool, and many studies show significant discordance between fasting glucose and HbA1c tests[36].

2.5. Factors Affecting HbA1c Level

There are many common factors, including genetic and medical conditions, that influence HbA1c and its measurement, even when glucose levels are constant[37,38]. Several studies have shown that in recent-onset, drug-naïve type 2 diabetes (controlling for age, sex and BMI), there is ethnic variability of HbA1c, despite similar fasting plasma glucose levels and similar or lower post-glucose load glucose levels[39,40]. People of African descent and South Asians appear to have significantly higher levels of HbA1c (and therefore levels "diagnostic" of diabetes) across the full spectrum of glycaemia[38], compared to Caucasians[41–43] the optimal HbA1c threshold for detecting diabetes may thus vary by ethnic group[44] while the WHO currently recommends a diagnostic HbA1c of 6.5%, other studies have shown that in some races a lower threshold of 6.3% can be used for detecting diabetes in high-risk populations[45]. In addition to the ethnic limitations described above, a number of other disorders influence HbA1c, and their frequencies will depend on the setting. Conditions that alter red cell lifespan alter the Hb1Ac concentrations, with conditions that shorten red cell survival, such as hemolysis, decreasing HbA1c, and those disease states that prolong red cell survival increasing HbA1C[46], owing to the change in the duration of contact of the red blood cells with glucose in the blood. Thus, decreased erythropoiesis caused by iron or vitamin deficiency may cause increased HbA1c; and increased erythropoiesis caused by hemolysis (for example, in malaria), administration of erythropoietin, iron, vitamin B12, and reticulocytosis, will lead to its decrease. Decreased lifespan of red blood cells resulting from haemoglobinopathies, splenomegaly (also common in malaria) rheumatoid arthritis, or drugs (for example, antimicrobials such as ribavirin and dapsone) can also decrease HbA1c. The relationship between HbA1c and HIV infection is also complex. Slama et al. found that at a fasting glucose of 125 mg/dL, median HbA1c values were 0.21% lower in HIV infected men than in HIV-uninfected men, and that the magnitude of this difference increased at higher glucose values[47]. They also found that HbA1c discordance was associated with lower cd4 cell counts; high mean corpuscular volume (mcv); high mean corpuscular haemoglobin; and a regimen containing a protease inhibitor, a non-nucleoside

reverse transcriptase inhibitor, and zidovudine[47]. The use of HbA1c could therefore lead to under diagnosis or under treatment of established diabetes mellitus, particularly in HIV positive people on antiretroviral therapy (art), or those with advanced disease[47]. This is significant since HIV and aids may have an impact on glucose metabolism, and there are an increasing number of people in Malawi who are dually affected by HIV and diabetes[48]. Finally, HbA1c testing (whether laboratory analysis or point of care) requires a rigorous program to standardize the assays[49], which may not be possible in many developing countries. Lack of standardization would lead to a high degree of uncertainty with the results generated in these settings[50].

2.6. Glycated Hemoglobin (HbA1c) for the Diagnosis of Diabetes

Glycated hemoglobin (HbA1c) was initially identified as an "unusual" hemoglobin in patients with diabetes over 40 years ago[51]. After that discovery, numerous small studies were conducted correlating it to glucose measurements resulting in the idea that HbA1c could be used as an objective measure of glycemic control. The A1C-Derived Average Glucose (ADAG) study included 43 participants representing a range of A1C levels. It established a validated relationship between A1C and average glucose across a range of diabetes types and patient populations[52]. HbA1c was introduced into clinical use in the 1980s and subsequently has become a cornerstone of clinical practice[53]. HbA1c reflects average plasma glucose over the previous eight to 12 weeks [54]. It can be performed at any time of the day and does not require any special preparation such as fasting. These properties have made it the preferred test for assessing glycemic control in people with diabetes. More recently, there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes[55]. Owing in large part to the inconvenience of measuring fasting plasma glucose levels or performing an OGTT, and day-to-day variability in glucose, an alternative to glucose measurements for the diagnosis of diabetes has long been sought. HbA1c has now been recommended by an International Committee and by the ADA as a means to diagnose diabetes[55]. Although it gives equal or almost equal sensitivity and specificity to a fasting or post-load glucose measurement as a predictor of prevalent retinopathy[56], it is not available in many parts of the world. Also, many people identified as having diabetes based on HbA1c will not have diabetes by direct glucose measurement and vice versa[51].

The relationship between HbA1c and prevalent retinopathy is similar to that of plasma glucose, whether glucose and HbA1c are plotted in deciles[57], in vicinities or as continuous variables. This relationship was originally reported in the Pima Indians[58] and has also been observed in several other populations including Egyptians[59], the NHANES study in the USA[60], in Japanese[61].

It is unclear whether HbA1c or blood glucose is better for predicting the development of retinopathy, but a recent report from Australia has shown that a model including HbA1c for predicting incident retinopathy is as good as or possibly better than one including fasting plasma glucose[62]. The use of HbA1c can avoid the problem of day-to-day variability of glucose values, and importantly it avoids the need for the person to fast and to have preceding dietary preparations. These advantages have implications for early identification and treatment which have been strongly advocated in recent years[51]. However, HbA1c may be affected by a variety of genetic, haematologic and illness-related factor[63]. The most common important factors worldwide affecting HbA1c levels are haemoglobinopathies (depending on the assay employed), certain anemias, and disorders associated with accelerated red cell turnover such as malaria[55,64]. The utility and convenience of HbA1c compared with measures of plasma glucose for the diagnosis of diabetes needs to be balanced against the fact that it is unavailable in many countries, despite being a recognized valuable tool in diabetes management. In addition, the HbA1c assay is not currently well enough standardized in many countries for its use to be recommended universally at this time. However, there will be countries where optimal circumstances already exist for its use. Factors influencing HbA1c assays are presented in Annex 2 and 3[51]. There are aspects of the measurement of HbA1c that are problematic. Although in some laboratories the precision of HbA1c measurement is similar to that of plasma glucose, global consistency with both assays remains a problem[55]. Whether it is the glucose or

HbA1c assay that is used, consistent and comparable data that meet international standards are required. This is starting to happen in many countries but obviously is still not standard across the world. Within any country, it is axiomatic that results for glucose and HbA1c should be consistent between laboratories[51].

The National Glyco hemoglobin Standardization Program (NGSP)[65] was established following the completion of the Diabetes Complications and Control Trial (DCCT). For many years it was the sole basis for improved harmonization of HbA1c assays. More recently the International Federation of Clinical Chemists (IFCC) established a working group on HbA1c in an attempt to introduce an international standardization program[66]. An important part of this effort was establishment of reference method procedures for HbA1c. Currently, both the NGSP and the IFCC base their evaluations on reference method procedures that have further enhanced the harmonization of HbA1c assays across manufacturers. Finally, in the USA, the College of American Pathologists (CAP) has mandated more stringent criteria for individual assays to match assigned values for materials provided in the CAP proficiency program[67]. A further major factor concerns the costs and availability of HbA1c assays in many countries. Also, the situation in several of these countries will be exacerbated by the high prevalence of conditions such as hemoglobinopathies, which affect HbA1c measurement, as discussed earlier[51].

A report published in 2009 by an International Expert Committee on the role of HbA1c in the diagnosis of diabetes recommended that HbA1c can be used to diagnose diabetes and that the diagnosis can be made if the HbA1c level is ≥6.5%[61]. Diagnosis should be confirmed with a repeat HbA1c test, unless clinical symptoms and plasma glucose levels >11.1 mmol/L (200 mg/dL) are present in which case further testing is not required. Levels of HbA1c just below 6.5% may indicate the presence of intermediate hyperglycemia. The precise lower cut-off point for this has yet to be defined, although the ADA has suggested 5.7–6.4% as the high risk range [68]. While recognizing the continuum of risk that may be captured by the HbA1c assay, the International Expert Committee recommended that persons with a HbA1c level between 6.0 and 6.5% were at particularly high risk and might be considered for diabetes prevention interventions[51].

3. Conclusion

International standardization of the HbA1c analysis has made it suitable not only for the follow-up of patients with diabetes, but also for diagnostic purpose. It should be simpler to identify diabetes at an early stage when HbA1c is used as the main diagnostic criterion. HbA1c appears to be a useful, convenient, and reliable tool for identifying subjects with prediabetes and diabetes and should be considered in the development of diagnostic strategies. HbA1c provides a number of benefits and doesn't require fasting, despite the fact that its use as a biomarker of average plasma glucose has significant limits, particularly in relation to erythrocyte turnover and metabolism. Unlike plasma glucose, which is unstable and requires time-consuming testing, it can be managed more easily. With HbA1c it should be used as the main diagnostic criterion. Diabetes is easier to identify in its early stages. It is important to promote early diabetes patient detection. Since successful late prevention depends on it problems with diabetes.

Conflict of Interest

The authors declare no conflict of interest.

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