

# Glycated Haemoglobin A1c in Contrast to Conventional and Novel Biomarkers in the Diagnosis of Diabetes and Monitoring of Glycemic Control: A Review

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## Abstract

Diabetes mellitus is an etiologically complex metabolic defect, characterized basically by an elevated blood glucose concentration. At present all countries across the globe, regardless of their developmental stages, face an increasing burden of diabetes mellitus. Diagnosis and monitoring of diabetes are done by many laboratory investigations. This review article discusses the potential of HbA1c in the diagnosis of diabetes and monitoring of glycemic control while looking into the characteristics of alternative novel markers of glycaemic control.

Available literature emphasized that HbA1c is the more accepted, accurate, easy-to-administer, confirmatory and reference test. The prognostic potential of HbA1c lies in its unique capability of assessing the retrospective glycemic control and predicting diabetic complications. However, the cut-off points of HbA1c, its sensitivity, some testing strategies, interferences and costs are heavily debated. Findings of the review conclude that the appropriate combinations of FBG, OGTT, HbA1c and novel alternative biomarkers of glycemic control, significantly enhances the diagnostic accuracy of these individual tests and such combination provide concrete information for more comprehensive diagnoses and effective treatment plans.

**Keywords:** : Glycated Haemoglobin, HbA1c, Diabetes Mellitus, diagnosis of diabetes, fructosamine, glycated albumin, 1,5-anhydroglucitol

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## Introduction

Diabetes mellitus is among the focal global public health concerns. It imposes a heavy burden to health and the economy of both developed and developing countries over the world. Several laboratory investigations are used for the diagnosis and monitoring of diabetes. Fasting Plasma Glucose (FPG) test and the Oral Glucose Tolerance Test (OGTT) possessed the lead of diagnosis and prognosis of diabetes, until the International Expert Committee made the recommendation that diabetes could be diagnosed by measuring haemoglobin A1c (HbA1c), in 2009. Subsequently, the World Health Organization endorsed the use of HbA1c with a cut off of  $\geq 6.5\%$  (48 mmol/mol) for the diagnosis of diabetes, in 2011. The objective of this review is to discuss the potential of HbA1c in the diagnosis of diabetes and monitoring of glycemic control and look into the alternative novel markers of glycaemic control.

## Methodology

We performed a literature review by searching Google Scholar, PubMed and Medline for original articles, review articles, systematic reviews, randomized control trials (RCTs) and meta-analyses published in the English language. A specific time period was not considered. A search string of medical subject headings (MeSH) including the terms 'glycosylated haemoglobin', 'HbA1c', 'diagnosis of diabetes', 'monitoring of diabetes', 'accuracy of HbA1c', 'diabetes with HbA1c', 'advantages of HbA1c', 'limitations of HbA1c', 'fructosamine', 'glycated albumin', and '1,5-anhydroglucitol'. The relevant articles were identified and manually reviewed for relevance with the context.

### Global trend of diabetes

Diabetes mellitus is a metabolic disease characterized by the elevated blood glucose levels. It is among the top ten global causes of premature death. It leads mortality and reduces the life

expectancy of the people around the world.<sup>[1]</sup> According to the International Diabetes Federation, at present 537 million adults (20-79 years) in the world are living with diabetes and the total number of patients with diabetes is projected to rise up to 643 million by the year 2030 and 783 million by 2045.<sup>[2]</sup> Diabetes has been a chief cause of kidney failure, stroke, ischaemic heart disease, blindness and lower limb amputation. According to World Health Organization (WHO), more than 95% of the people with diabetes have type 2 diabetes mellitus. There had been about 9 million people with type 1 diabetes mellitus in the world by 2017 and the majority of them were from high-income countries.<sup>[3]</sup> Globally, 18.4 million pregnancies are attributed to gestational diabetes mellitus. The prevalence of diabetes is growing faster in low- and middle-income countries compared to high-income countries. The global charge of diabetes has been valued to increase from \$1.3 trillion in 2015 up to between \$2.1 and \$2.5 trillion by the year 2030.<sup>[4]</sup>

### Diagnosis of diabetes

Numerous laboratory investigations are used for the diagnosis and monitoring of the patients with diabetes. Until 1979, diabetes was solely diagnosed by the direct identification of hyperglycemia, as measured by increased plasma glucose concentrations.<sup>[5]</sup> In 1979, considering the distribution of glucose concentrations in high-risk populations, a set of criteria was established in order to standardize the diagnosis and they were endorsed by the WHO. In 1997, the diagnostic criteria were modified including, a FPG level of  $\geq 7.0$  mmol/L (126 mg/dL); a 2-hour post-load glucose concentration  $\geq 11.1$  mmol/L (200 mg/dL) during an OGTT; or symptoms of diabetes and a casual (i.e., regardless of the time of the preceding meal) plasma glucose concentration  $\geq 11.1$  mmol/L (200 mg/dL).<sup>[5]</sup> When any of the above three criteria is encountered, a repeat testing on the subsequent day is mandatory to establish the diagnosis (this is not necessary for those who have unequivocal hyperglycemia, i.e.,  $>11.1$  mmol/L (200 mg/dL) with symptoms of hyperglycemia). WHO and the International Diabetes Federation (IDF) have recommended either FPG test or 2-h post-load glucose test with the same cut-offs for the diagnosis of diabetes. In 2009, International Expert Committee made the recommendation that type 2 diabetes should be diagnosed by measurement of haemoglobin A1c (HbA1c), based on the fact that it better reflects the long-term blood glucose concentrations of the patient.<sup>[6]</sup> WHO authorized the use of HbA1c with a cut off of  $>6.5\%$  for the diagnosis of diabetes in 2011, provided that “stringent quality assurance tests are in place and assays are standardized to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement”. However, according to WHO, a HbA1c value of less than 6.5% should not exclude diabetes diagnosed using glucose tests.<sup>[7]</sup>

### Monitoring of diabetes

A moment-in time snapshot of glucose levels of an individual could be obtained by fasting or non-fast

ing blood glucose tests. Blood glucose levels of a person fluctuate substantially over the course of hours and days. Therefore, blood glucose testing does not provide a good insight for the long-term glycemic control. HbA1c is less affected by day-to-day differences in blood glucose levels, in comparison to fasting plasma glucose and glucose tolerance tests.<sup>[8]</sup> Importantly, HbA1c has the ability to indicate the cumulative glycemic history of the past 90 to 120 days. Therefore, it provides more precise information over glycemic control as a reliable measure of chronic hyperglycemia.<sup>[9]</sup> Further, HbA1c has found to correlate well with the risk of long-term complications of diabetes. Elevation of HbA1c is also regarded as an independent risk factor for coronary heart disease and stroke. Hence a single HbA1c test is considered as a reliable biomarker for monitoring of diabetes.<sup>[9]</sup>

### What is HbA1c?

The term ‘HbA1c’ is referring to the glycated haemoglobin. Haemoglobin is an oxygen-binding protein, found in erythrocytes, which transports oxygen from the lungs to the tissues.<sup>[10]</sup> HbA1c is formed by the condensation of glucose with the N-terminal amino groups of the beta-chains of haemoglobin A (adult haemoglobin/  $\alpha_2\beta_2$ ) even though this only accounts for approximately half of the entire glycation of the haemoglobin molecule.<sup>[11]</sup> Glucose binds with haemoglobin by a non-enzymatic reaction which begins with an Amadori molecular rearrangement reaction through Schiff base aldimine intermediates. Between glucose and haemoglobin molecule an unstable bond is initially formed and later it rearranges to form a more stable compound in which glucose is covalently bound to haemoglobin. The concentration of the unstable form increases rapidly when blood glucose level is elevated. The stable form changes slowly and provides a time-average integral of the blood glucose concentration through the 120-day life span of the red blood cell. Therefore, the level of glycohaemoglobin in blood provides an objective measurement of average diabetic control over time.<sup>[12]</sup> About eight decades ago, HbA1c was initially discovered as an ‘unusual’ haemoglobin in patients with diabetes.<sup>[13]</sup>

HbA1c was first isolated historically by Huisman et al. in 1958<sup>[14]</sup> and characterized as a glycoprotein by Bookchin and Gallop in 1968.<sup>[15]</sup> The raised-up levels of HbA1c in patients with diabetes were reported by Rahbar et al. in 1969.<sup>[16]</sup> Since this discovery, multiple studies have been conducted to correlate the level of HbA1c with the blood glucose measurements, with the view of exploring the possibility of using HbA1c as an objective measurement for glycemic control. As a result, in 1980s, HbA1c was introduced into clinical practice and afterward it became a cornerstone in clinical practice, both as a diagnostic biomarker to identify patients with diabetes and as a pharmacodynamic/response biomarker of DM.<sup>[17]</sup> The normal lifespan of a red blood cell is approximately 4 months. The amount of HbA1c synthesized is directly related to the average blood glucose concentration of an individual over the previous 8-12 weeks. Therefore, HbA1c became the

preferred test for monitoring long-term glycemic control and the 'gold standard' for assessing the treatment in both types of DM. Further, HbA1c is considered as a validated surrogate endpoint regarding the reduction of microvascular complications, associated with DM.<sup>[18]</sup>

### How to measure HbA1c levels in blood?

At present four types of methods are commonly used to measure the levels of HbA1c in blood. They include ion-exchange high-performance liquid chromatography (HPLC), boronate affinity HPLC, immunoassay and enzymatic assays. Immunoassays measure HbA1c through antibodies identifying the N-terminal glycated amino acids structure, typically the first 4-10 amino acids of the  $\beta$  chain of haemoglobin. In Ion-exchange HPLC different haemoglobin species are separated based on the differences of the charge between HbA1c and other haemoglobin molecules. Both these methods are variably susceptible to the presence of Hb variants and derivatives. In the boronate affinity technique, m-aminophenylboronic acid specifically reacts with the cis-diol groups of glucose bound to Hb and this method measures total glycated haemoglobins, including HbA1c and Hb glycated at other sites and therefore it tends to be less affected by haemoglobinopathies or Hb derivatives. Currently available enzymatic methods measure the HbA1c levels by using an enzyme that specifically cleaves the N-terminal valine.<sup>[19]</sup> Out of these methods the enzymatic assay is the most widely used technique to measure HbA1c in healthcare settings around the world as it is comparatively less expensive and faster.

### Comparability of HbA1c with glucose measurement

Chronic hyperglycemia is the biochemical hallmark of diabetes. A few decades ago, diabetes was principally diagnosed by the measurement of fasting plasma glucose or sometimes by an oral glucose tolerance test.<sup>[20]</sup> However, FPG and OGTT reflect the glycemic status of just a moment of a particular day and therefore they detect spot hyperglycemia. They therefore may not reflect the chronic glycemic status of an individual. Declaring an individual as having diabetes can carry a lot of healthcare, psychological and legal implications. Therefore, a robust approach is mandatory in this regard. It is accepted that the measurement of HbA1c is comparable to the assessment of FPG and postprandial glucose peaks.<sup>[20]</sup> Therefore, HbA1c is potentially a more convenient alternative to measurement of FPG and 2-h OGTT.

Rathod et al have examined the ability of HbA1c to detect glucose-defined type 2 diabetes and impaired fasting glucose among a large population of adults (n=3645) living in Malawi, in 2018. Results of this study reported that HbA1c proved dependable in detecting FPG-defined diabetes, with an area under the receiver operating characteristic curve of 0.92 (95% CI 0.90 to 0.94). At HbA1c  $\geq 6.5\%$  (140 mg/dL), the sensitivity has been 78.7% and specificity has been 94.0%. These findings have provided good recent confirmation in using HbA1c to detect type 2 diabetes.<sup>[21]</sup>

Khan et al. have studied the validity of HbA1c cut-point of 6.5% for diagnosis of diabetes in 2014. This study included 12785 male patients with diabetes (FPG  $\geq 7.0$  mmol/L, mean age  $56.27 \pm 13.32$ ) from Saudi Arabia. The mean FPG and HbA1c levels of the sample were  $10.127 \pm 0.026$  mmol/L and  $8.729 \pm 0.013\%$ , respectively. This study has reported a significant correlation ( $R=0.571$ ,  $p<0.001$ ) between FPG and HbA1c. However, as there has been 3.78% false negative predictions (with borderline FPG of 7.0-8.0 mmol/L and HbA1c 6.0-6.5%), it has suggested that Saudi individuals with HbA1c between 6.0% and 6.5% could be considered as probable diabetes.<sup>[22]</sup>

Hazeeb et al has conducted a regression analysis on 75 patients with diabetes in Saudi Arabia in 2015 to test the association between fasting blood sugar (FBS) and HbA1c and attempted to postulate regression equations for inter-conversion of their levels. The average FBS and HbA1c levels reported among this sample were  $8.101 \pm 3.917$  mmol/l &  $7.989 \pm 2.112\%$ , respectively. Patients with HbA1c  $>6.5\%$  have had significantly higher levels of FBS. This study has also reported a significant correlation between FBS and HbA1c ( $r=0.717$ ,  $p<0.001$ ). Further the regression equations,  $HbA1c=0.387 (FBS) + 4.855$  and  $FBS=1.33 (HbA1c) - 2.528$  have formulated by the results to be utilized for inter-conversions between the FBS and HbA1c levels for predicting expected values in patients with diabetes.<sup>[23]</sup>

### Complication detection and prognostic potential of HbA1c

Diabetes mellitus is associated with multiple microvascular and macrovascular complications. HbA1c is not only important to assess the long-term glycemic control but also to assess the risk of diabetic complications. Numerous clinical studies have reported that high HbA1c levels are associated significantly with the risk of long-term diabetes-related complications. Diabetic retinopathy has been reported to be increased with A1C levels above 6.5%.<sup>[24-26]</sup>

Colagiuri et al have evaluated the relationship between glycemia and diabetic retinopathy by conducting a data-pooling analysis. This analysis has included nine studies from five countries with 44,623 participants (20-79 years old) with gradable retinal photographs. A curvilinear relationship has been observed for FPG and HbA1c, when diabetes-specific retinopathy was plotted against continuous glycemic measures. Diabetes-specific retinopathy prevalence has been low for FPG  $<6.0$  mmol/l and A1C  $<6.0\%$  but increased above these levels. Further the glycemic thresholds for diabetes-specific retinopathy have been observed above the range of 6.4-6.8 mmol/l for FPG, 9.8-10.6 mmol/l for 2-h PG, and 6.3-6.7% for HbA1c. Thresholds for diabetes-specific retinopathy from receiver-operating characteristic curve analyses had been 6.6 mmol/l for FPG, 13.0 mmol/l for 2-h PG, and 6.4% for HbA1c. These scientific analyses have suggested that the current diabetes diagnostic level for FPG could be lowered to 6.5 mmol/l and that HbA1c of 6.5% is a suitable



alternative diagnostic criterion.<sup>[27]</sup>

Cardiovascular disease (CVD) is the most frequent chronic complication of diabetes. CVD is reported in patients with diabetes with incidence rates 5 to 10 folds higher than with microvascular disease. Evidences have indicated that FPG is a poor marker of future CVD events, while HbA1c is a good predictor.<sup>[28-29]</sup>

HbA1c has reported as a good predictor of lipid profile. Hence glycemic control monitoring using HbA1c provides added advantages of identifying diabetes patients who are at a greater risk of cardiovascular complications.<sup>[30]</sup> A study conducted with 1,011 type 2 diabetic patients has reported that HbA1c indicated direct correlations with lipid profile parameters including cholesterol, triglycerides, and low-density lipoprotein cholesterol and inverse correlation with high-density lipoprotein cholesterol. Further, there had been a linear relationship between HbA1c and dyslipidemia in patients with worse glycemic control as compared to patients with good glycemic control.<sup>[30]</sup>

The results of a community-based population study conducted on non-diabetic patients (n=11092) has reported that elevated HbA1c level is strongly associated with the risk of cardiovascular disease and mortality.<sup>[31]</sup> Further, elevated levels of HbA1c have been associated with an increased risk of recurrence of atrial tachyarrhythmia in patients with type 2 DM and paroxysmal atrial fibrillation undergoing catheter ablation.<sup>[32]</sup>

Another important advantage of HbA1c as a biomarker for glycemic control is that it can be used for both diagnosis and monitoring the prognosis of the patients. Changes of the targets of HbA1c levels prompt physicians to alter treatment plans with drug modifications and or lifestyle interventions. In high-risk patients with diabetes who are with HbA1c of 6.00-6.49%, an effective prevention strategy can be immediately started using a single HbA1c with a higher reliability than a single FPG.<sup>[20]</sup>

### Convenience and minimum perturbations

HbA1c test does not require any specific patient preparation prior to the collection of blood. No fasting is required, so that blood samples can be collected at any time of the day. Obtaining a single blood sample is adequate for the test. HbA1c is a much more stable analyte than glucose, hence stringent requirements for rapid processing, separation and storage are not mandatory.<sup>[7]</sup> It is well known that diet, stress, exercise, smoking and even certain medication substantially affect the FPG. In contrast HbA1c is not influenced by any of the above perturbations, at least acutely.<sup>[20]</sup>

### Analytical standpoints

Even under optimal patient preparation conditions the blood glucose values could be misleading due to greater pre-analytical instability. Glucose concentration of a blood sample decreases 5–7% per hour and even more rapidly under high ambient

temperature.<sup>[33]</sup> Even with antiglycolytic substances, a significant glucose consumption occurs in blood cells during the first 1–2 h of sampling, since glycolysis is inhibited in its more distal steps by sodium fluoride or other preservatives. The pre-analytical variability of FPG has been estimated to be 5–10%. In contrast the pre-analytical variability of HbA1c is negligible.<sup>[20]</sup>

The biological variability of HbA1c is also much lower than with FBG. This means, if two assessments are made on the glucose-related parameters, the correlation is stronger among the individual HbA1c measurements than among the FPG or 2-h post glucose measurements. The coefficients of variation of HbA1c, FPG, and 2-h post glucose are reported to be 3.6, 5.7, and 16.6%, respectively.<sup>[34]</sup> These coefficients reflect both biological and analytical variability. However, the biological variability of HbA1c is several fold lower than that of FPG.<sup>[35]</sup> This fact confirms that two assessments of FPG to diagnose diabetes could provide untrustworthy information, whereas HbA1c, if measured twice would definitely offers more strong clinical information.

Poor standardization of the assay remained as one of the main concerns behind the use of HbA1c in the diagnosis of diabetes. Hence, an large effort has been made by clinical and laboratory staff in the United States and many other countries to improve the comparability of HbA1c results across laboratories with the use of an effective standardization programme, which has now been implemented worldwide.<sup>[36]</sup> The said standardization programme has minimized between-laboratory biases. It has been a prerequisite to using HbA1c for both diagnosing and reliably monitoring diabetes. The general belief is that glucose assays are highly reproducible across laboratories. However, this is not necessarily true. A survey conducted in 6,000 U.S. laboratories have clearly reported a significant bias in glucose measurement in 41% of them, producing a mis-classification of glucose tolerance in 12% of individuals.<sup>[37]</sup> Hence, the argument that HbA1c cannot be used for diabetes diagnosis due to poor standardization is no longer defensible.<sup>[20]</sup>

### Pitfalls of HbA1c test in the diagnosis of diabetes

Diabetes is a clinical condition of an increased blood glucose concentration. High levels of HbA1c characterizes high protein glycation in the body. It is a markedly different biochemical anomaly, which is secondary to increased blood glucose. In good medicine, it is always important to pay attention to primary indications before paying attention to the secondary ones. Elevated HbA1c is only seen subsequently to an increase in blood glucose. Because of this delay, diagnosis of diabetes using HbA1c might take place later than with blood glucose measurements. Such a delay could lead to negative clinical consequences in many individuals.<sup>[20]</sup>

Unlike the OGTT and 2-h post-glucose, HbA1c is a poor marker for underlying pathophysiology behind diabetes. HbA1c captures only chronic

hyperglycemia, but it will not provide any information about acute hyperglycemia, capacity of the pancreatic  $\beta$  cells, how well the body could respond to a given glucose load, insulin secretion or insulin resistance etc. In contrast, 2-h OGTT provides reliable information about the key pathophysiological defect of diabetes, providing guidance on the correct therapy to overcome it. The Insulin Resistance Atherosclerosis Study (IRAS) has proved that HbA1c is a weaker correlate of insulin resistance and insulin secretion compared with FPG and 2-h PG.<sup>[38]</sup>

Poor sensitivity is the other major limitation of HbA1c in the diagnosis of diabetes. Sensitivity of HbA1c to detect diabetes defined by the OGTT has been found to be 50%.<sup>[20]</sup> Therefore, half the patients with glucose-defined diabetes would remain undiagnosed if HbA1c is used solely. The worst part of the story is that the individuals with impaired glucose tolerance who are proven to have the capacity to get prevented from diabetes may not be detected by HbA1c. A large number of population-based studies conducted on different ethnic groups have proven that the WHO cut off point of HbA1c  $\geq 6.5\%$  is not fitting for the diagnosis of diabetes. Chivase et al assessed the diagnostic accuracy of HbA1c, compared to FBG and OGTT, in screening for type 2 DM in Africa in 2021, by a systematic review and meta-analysis. This study has included eleven studies, conducted seven African countries, enrolling 12,925 participants. Compared to OGTT, HbA1c  $\geq 48$  mmol/mol (6.5%) have shown a pooled sensitivity of 57.7% (95% confidence interval [CI] 43.4-70.9) and specificity of 92.3% (95% CI 83.9-96.5). Compared to FPG, HbA1c  $\geq 48$  mmol/mol (6.5%) had a pooled sensitivity of 64.5% (95% CI 50.5-76.4) and specificity of 94.3% (95% CI 87.9-97.5). The highest sensitivity for HbA1c, against the OGTT, has been at the 42 mmol/mol (6.0%) cut-off. Their findings have concluded that the HbA1c  $\geq 48$  mmol/mol (6.5%) cut-off may miss almost half of the individuals in Africa with type 2 DM based on blood glucose measures.<sup>39</sup> Shibata et al have also compared the sensitivity, specificity, and accuracy of HbA1c of  $\geq 6.5\%$  in the detection of hyperglycemia in contrast to FBG of  $\geq 7.0$  mmol/L in a total of 6,010 subjects in Japan. This study has calculated the true and false positive odds ratios to evaluate the sensitivity and specificity of HbA1c relative to FBG, and compared the overall accuracy calculating the conditional relative odds ratio (CROR). According to their results the true and false positive odds ratios have been 0.43 (95% CI 0.26-0.69) and 0.40 (0.13-1.27) (Fisher's exact test,  $p < 0.000$ ), respectively; the CROR was 1.07 (95% CI 0.30-3.75). These findings have concluded that HbA1c test is marginally more specific, however less sensitive than the FBG, at given cut-off points.<sup>[40]</sup> Amaefule et al conducted a meta-analysis regarding screening and diagnostic accuracy of HbA1c test in women with and without risk factors for gestational diabetes. This study has pooled data of 16921 women, using a multiple thresholds model. Their findings have

concluded that HbA1c is more useful as a specific test at a cut-off of 5.7% with a false positive rate of 10%, and it is recommended to be supplemented by a more sensitive test to detect gestational DM.<sup>[41]</sup> Another trouble with HbA1c is that the test is less reliable to apply on all individuals. HbA1c levels are differ not only based on the glycemia, but also to turnover rates of the red blood cells. Therefore haemoglobinopathies, malaria, chronic anemia, major blood loss, hemolysis, uremia, pregnancy, smoking, infections, aging, as well as some other factors could affect the HbA1c levels. Abnormal haemoglobin traits are also not uncommon. They interfere significantly with HbA1c test, producing false results. Therefore, until different cut points are introduced regarding all above conditions HbA1c cannot be simply used to diagnose diabetes.<sup>[20]</sup>

Cost of the test is also another main shortcoming. HbA1c assay is highly expensive compared to glucose assays. In most of the countries in the world, HbA1c is not available, and its cost is too high. It looks like already the world has unintentionally divided into two categories; developed and rich societies who diagnose diabetes with HbA1c and less developed and poor societies who diagnose diabetes using FPG, producing an obvious inequity in global health and health care.<sup>[20]</sup>

Further, the standardization of HbA1c assay is sub-optimal, even in Western countries in the world up to date. In contrast, standardization of glucose assays is much easier and inexpensive. Though proper standardization programmes are introduced, still there is a long road ahead to a global standardization of the HbA1c assays.<sup>[20]</sup>

### **Pitfalls of HbA1c test in the monitoring of diabetes**

HbA1c is a guideline-recommended test for monitoring the patients with diabetes. However, the HbA1c levels of a follow-up patient with diabetes could be affected by various factors. Factors which extend the lifespan of red blood cells or decrease rate of red blood cell turnover increase the exposure of red cells to glucose. This leads to falsely high HbA1c results.<sup>[42]</sup> Such situations may occur in iron, folate and B12 deficiency anemias, in chronic alcoholics, and in asplenia.<sup>[43]</sup> On the other hand, some conditions which shorten the lifespan of red blood cells bring about reduced exposure of cells to glucose with falsely low HbA1c results. This situation has been reported in hemolytic anemia, acute/chronic blood loss and in pregnancy.<sup>[43]</sup> Diabetes patients are susceptible to the development of kidney diseases. Chronic kidney disease (CKD) is a challenge for measuring HbA1c. This occurs specially when the glomerular filtration rate reaches below 30 mL/min per 1.73 m<sup>2</sup>.<sup>[44]</sup> Such patients develop uremia and anemia of multifactorial origin. Uremia causes the production of carbamylated haemoglobin which interferes with HbA1c measuring techniques.<sup>[45]</sup> CKD patients are frequently treated with erythropoietin and this has also found to produce falsely low HbA1c results. Hence it is recorded that CKD produce unreliable HbA1c measurements.<sup>[42]</sup>

## Novel markers of glycemic control

At present there is a rising attention towards alternative biomarkers for glycemic control. Among these are fructosamine, glycated albumin (GA) and 1,5-anhydroglucitol (1,5-AG).<sup>[46]</sup>

Fructosamine refers to glycated serum proteins dominated by albumin. It is formed by non-enzymatic glycation of amino groups in proteins via a similar process by which HbA1c is formed. Albumin has a shorter half-life of approximately 20 days. Therefore, fructosamine reflects more short-term (2-3 weeks) changes in glycemic control than HbA1c. Affinity chromatography, high performance liquid chromatography and colorimetric enzymatic methods are employed to determine the serum fructosamine levels.<sup>47</sup> Fructosamine assays are generally easier to perform and cheaper than HbA1c assays. However, to date, none of these assays are popular as routine laboratory assays. The main advantage of fructosamine is that it is beneficial in patients with haemoglobin variants (which interfere with HbA1c assays), for the individuals who are suffering from diseases that affect the ordinary lifespan of red blood cells (such as thalassemia), for the patients with diabetes who undergo multiple treatment changes and for the pregnant women.<sup>[48]</sup>

GA refers to the proportion of serum GA to total albumin. Though it is similar to serum fructosamine, it is not influenced by the serum albumin levels. As the half-life of albumin is shorter than red blood cells, GA reflects the glycemic control over 2-3 weeks which is shorter than that of the HbA1c.<sup>47</sup> Both GA and fructosamine have proven to be strongly associated with HbA1c and FBG.<sup>[49]</sup> Advantages of GA include an accurate assessment of recent glycemia, being unaffected by red cell lifespan or variant haemoglobin, applicability in hematologic disorders (e.g., anemia, hemorrhage, renal anemia, pregnancy) and in neonatal DM, usefulness for conditions where the glycemia improves or deteriorates swiftly.<sup>[50]</sup> However, GA is reported to produce discrepant results in subjects with abnormal albumin metabolism (e.g., nephrotic syndrome, hyperthyroidism, hypothyroidism, glucocorticoid administration, Cushing's syndrome, liver cirrhosis), in infants and in obesity.<sup>[46]</sup>

1,5-AG is the 1-deoxy form of glucose. It is a naturally occurring dietary polyol. Under normo-glycemia, all the serum 1,5-AG are reabsorbed at the renal tubules. Therefore, serum 1,5-AG concentrations of healthy individuals are kept at a constant steady state (approximately 12-40 µg/mL). When blood glucose level is elevated, serum 1,5-AG competes with glucose for reabsorption. This results in an increased urinary loss and a fall in circulating 1,5-AG level.<sup>[51]</sup> Thus, lower serum 1,5-AG levels indicate high levels of circulating glucose and the occurrence of glycosuria over the past 1-2 weeks. Serum 1,5-AG is reported to reflect postprandial glycemic excursion better than HbA1c.<sup>[52]</sup> 1,5-AG has been accepted to have better clinical implications in the evaluation and treatment of glycemic excursions in type 1 DM. Renal

sodium-glucose co-transporter-2 (SGLT2) inhibitors are now being popular in the treatments of type 2 DM. Their mechanism of action is by increasing the urinary glucose excretion. SGLT2 enhance the reabsorption of 1,5AG and thereby decrease the plasma 1,5AG levels. A recent Japanese study has reported 1,5-AG as the most reliable indicator for predicting HbA1c reduction in type 2 DM patients who treated with SGLT2 inhibitors.<sup>[53]</sup> However, 1,5-AG test is disturbed by alterations in renal hemodynamics.<sup>[46]</sup>

## Conclusion

Diabetes mellitus is among the leading global causes of premature death. Accurate and timely diagnosis and proper monitoring is obligatory for efficient patient care and to control the healthcare burden. HbA1c is a reliable test to help achieve these goals. The prognostic potential of HbA1c lies in its capability of evaluating retrospective glycemic control plus predicting many diabetic complications. Nevertheless, while HbA1c has been endorsed for diagnosis of diabetes and evaluation of prognosis worldwide, its cut-off points, inadequate sensitivity, some testing strategies, haemoglobin variant interferences and costs are still being hotly debated.

Available literature suggests there may be a role for the appropriate combinations of FBG, OGTT, HbA1c and novel alternative biomarkers of glycemic control to enhance the clinical utility of these individual tests. Such combinations could potentially provide additional information for a more comprehensive diagnosis and for effective treatment plans. Until adequate epidemiological and clinical data are produced to make strong evidence-based consensus, complete replacement of FBG with HbA1c may not be appropriate or desirable for many clinical settings.

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