Review Article:

Clinical applications of glycosylated haemoglobin

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ABSTRACT

Glycosylated haemoglobin (HbA1c) has been in use since 1980s as the 'gold standard' for monitoring glycaemic control and as a predictor of diabetic complications. Even though several conditions, such as, haemolytic anaemia (lowers HbA1c) and aplastic anaemia (raises HbA1c) tend to confound and interfere with HbA1c measurement, in most circumstances HbA1c is a valid and reliable index of glycaemic status. Recently, HbA1c has also been recommended as a diagnostic test for diabetes mellitus by the American Diabetes Association (ADA); HbA1c offers logistical advantages over the conventional oral glucose tolerance test as it requires a non-fasting random sample. In this article the history of discovery of HbA1c, biochemical processes behind its formation, its assay techniques, various factors influencing HbA1c, importance of standardization of its assay so as to make the results reported from different laboratories much more comparable are critically reviewed. This review also provides an update on the optimal HbA1c targets, its reliability in control of diabetic complications, limitations of test results and its importance in control of diabetes patients and their complications, various cut-off values obtained in studies performed both in India and worldwide and its role as a surrogate marker of metabolic syndrome, among others.

Key Words: Haemoglobin A, Glycosylated, HbA1c, Biosynthesis, Standards, Diagnostic use

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INTRODUCTION

Glycosylated haemoglobin (HbA1c) is on its way to celebrate 50 years of existence and is being considered as one of the best achievements in the history of diabetes mellitus (DM). Over the last five decades, HbA1c has emerged as a marker of glycaemic control, glycaemic risk, predictor of diabetic complications and as a screening tool for diagnosis of DM and metabolic syndrome, among others.

HISTORY

It was way back in 1962, Huisman and Dozy[1] reported an increase in one of the minor fractions of haemoglobin in four of their diabetic patients who were on oral hypoglycemic drug tolbutamide and they attributed the hike to the intake of the drug. However, they failed to reproduce this phenomenon in vitro. After five years of documenting this unusual finding, HbA1c was described as "an abnormal haemoglobin in diabetes" by Samuel Rahbar.[2] In the process of screening for the presence of abnormal haemoglobins in persons with DM, Rahbar,[2] demonstrated that a normal fraction of haemoglobin A1 was found in slightly higher amounts in their blood. Further investigations in patients with poorly controlled DM resulted in the finding of a "diabetic haemoglobin component" that was reported in 1968.[3] Later on, several structural studies were carried out in patients with DM and that so called "abnormal haemoglobin" was found to be identical to HbA1c fraction. Furthermore, this "abnormal haemoglobin" was found to be increased in direct proportion to the degree of hyperglycaemia.[4-6]

Significance of HbA1c in monitoring blood glucose control in patients with DM was proposed by Cerami and Koenig in 1976.[7] In the 1980s, HbA1c evolved as a better index of glycaemic control in clinical trials.[7,8] This, along with the other method that emerged by that time, namely, self-monitoring of blood glucose (SMBG) greatly enhanced the achievement of glycaemic control. Regular SMBG had a positive effect on improving glycaemia especially in individuals treated with

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insulin. SMBG reflects the immediate plasma glucose levels, whereas HbA1c measures long-term glycaemic control.[9] After the data from The Diabetes Control and Complications Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) became available, HbA1c has become an integral part of monitoring the glycaemic control in DM. The American Diabetic Association (ADA) recommendation of the goal of achieving a HbA1c level of less than 7 as evidence of satisfactory glycaemic control in patients treated for DM revolutionized the significance of HbA1c as a diagnostic test for assessing the adequacy of glycaemic control.

FORMATION OF HbA1c

The normal lifespan of an erythrocyte is 120 days. In the presence of hyperglycaemia, as the erythrocyte circulates, the N-terminal valine residues of β chain of haemoglobin gradually undergoes nonenzymatic glycation.[10] The HbA1c thus formed, constitutes about 60% to 80% of the total glycated haemoglobin. The number "1c" represents the order of haemoglobin detection on chromatography. The glycation of haemoglobin occurs over the entire 120-day life span of erythrocyte.[11] The glycation process follows a peculiar pattern. The initial 25% of glycation occurs in the first 1 to 2 months of the life span of the erythrocyte, another 25% occurs in the next month. The remaining HbA1c is formed during the senenscence of the erythrocyte corresponding to the period of 1-2 months prior to measurement. Consequently, older, senescent erythrocytes have more HbA1c than the reticulocytes. Furthermore, this forms the rationale behind the assumption that HbA1c represents average glycaemia over the last 6 to 8 weeks.[11-13]

Due to the post-translational, post-secretory glycation, an unstable aldamine-Schiff base is formed, which is a reversible process. This slowly undergoes an Amadori rearrangement to form a stable irreversible ketoamine linkage, which is an advanced glycation end-product. The reaction is essentially irreversible, meaning that once the haemoglobin molecule becomes glycated it remains so until the end of its lifespan. While the senescent erythrocytes lose their ability to metabolize glucose, they remain permeable to glucose. Thus, the intracellular glucose concentrations reflect the extracellular glucose concentrations. The clinical assay of HbA1c measures total glycation of haemoglobin: i.e., it measures glycation of haemoglobin in both less glycated young erythrocytes as well as more glycated senescent erythrocytes.

FACTORS AFFECTING HbA1c

As the level of HbA1c depends upon the lifespan of erythrocyte, the most important factor that influences the HbA1c level is erythrocyte turnover rate. Therefore, longer the erythrocyte circulation time, the more glycated its haemoglobin becomes. Falsely elevated HbA1c concentrations can be encountered when there is increased circulating erythrocyte life span (i.e., decreased red cell clearance) or impaired reticulocyte production. In older erythrocytes, haemoglobin is likely to have had a longer period of exposure to hyperglycaemia and this results in the formation of higher HbA1c levels. Some of the well documented causes for elevated HbA1c include alcoholism, iron deficiency, renal failure, and hyperbilirubinaemia.[8]

Any condition that shortens the life span of erythrocytes is likely to decrease HbA1c levels, since the average erythrocyte is younger, lasting for lesser time in circulation to be glycated. Falsely decreased HbA1c values are seen in conditions with a reduced erythrocyte life span (i.e., increased haemoglobin turnover) or where a large number of reticulocytes are produced. These younger erythrocytes have less time exposure to ambient glycaemia. Well known causes that result in this condition include acute or chronic blood loss, sickle cell anaemia, thalassaemias, glucose-6-phosphate dehydrogenase (G6PDH) deficiency, haemolytic, aplastic anaemias, and splenectomy. Pregnancy may falsely increase or decrease HbA1c, suggesting that this investigation is not to be considered appropriate for the diagnosis of

gestational diabetes mellitus.[8] In case of aplastic anaemias, cessation of erythropoiesis leads to an increase in the number of circulating aged red cells resulting in a progressive rise in HbA1c.

Effect of abnormal haemoglobins

The normal phenomenon is the glycation of adult HbA0 to form HbA1c. However, when abnormal haemoglobins are present, the person is likely to form other glycated products such as HbS1c, HbC1c, and so on, either in addition to or instead of HbA1c.[14] In some persons persistence of foetal haemoglobin can cause considerable problem in measuring HbA1c as they would co-migrate or co-elute with the HbA1c fraction leading to an overestimation of the HbA1c levels.[15]

Effect of anaemia

Iron deficiency anaemia has been known to cause a rise in HbA1c of up to 2% and this has been shown to be reversed with iron supplementation.[16-19] Given that iron deficiency anaemia is a common finding, especially in pre-menopausal women, caution should be exercised while interpreting HbA1c results in these patients. Haemolytic anaemia has the opposite effect to iron deficiency and a reduction in HbA1c is observed in affected individuals. This occurs due to reduced red cell survival, meaning a reduction in the availability of haemoglobin for glycation.[20]

Effect of drugs and health conditions

Any drug which gives rise to haemolytic anaemia will result in lowering of HbA1c levels. High-dose aspirin, by forming acetylated haemoglobin, can lead to spurious rise in HbA1c when certain methods are used for estimation, but the effect is usually only apparent at doses (4 g/day)that are well in excess of that prescribed normally.[21] Chronic kidney disease (CKD) can have complex influences on HbA1c formation and measurement. Patients with CKD can be iron deficient, exhibit haemolytic anaemia and have altered red cell survival, all of which influence HbA1c level. Compounding the problem is the fact that urea-derived isocyanate can lead to the formation of carbamylated haemoglobin, which can be indistinguishable from HbA1c when using certain HbA1c assay methods.[21]

Differences in HbA1c can also occur due to potential racial and ethnic differences.[22-24] African Americans have higher HbA1c levels than Caucasian whites . This difference accentuates as glucose intolerance worsens. In these ethnic groups, these significant limitations affect all the possible applications of HbA1c , i.e., to screen for glucose intolerance, to assess the risk for complications, to measure quality of care, and to evaluate disparities in health.

ASSAYS TO MEASURE HbA1c

Till 1999, the following assays were being used for measuring HbA1c were boronate affinity chromatography (more than 50% of laboratories), cation or ion-exchange high performance liquid chromatography (HPLC) methods (30%), immunoassay (15%), and electrophoretic methods (<5%).[25] However, presently, cation exchange performed by HPLC is the most widely used assay method.[26]

HbA1c accelerates faster in a cation-exchange resin. Ion exchange chromatography takes advantage of the lower isoelectric point that develops when glucose attaches to the β-chain N-terminal valine and HbA1c acquires an extra negative charge. The concentration of haemoglobin is measured using a spectrophotometer and quantified by calculating the area under each peak of the chromatogram compared with a calibrated chromogram.[27] The BioRad Diamat (HPLC cation exchange using Bio-Rex 70 resin; BioRad, Hercules, CA, USA) was the reference method used for the DCCT; the MonoS assay (Pharmacia Biotechnology, Uppsala, Sweden) and KO500 (Japanese Society for Clinical Chemistry) are also ion-exchange HPLC systems.[28]

There is no question that different methods and laboratories yield different HbA1c results. This has caused major comparability problems and the need for standardization which has been emphasized by

many diabetes organizations. The National Glycohaemoglobin Standardization Program (NGSP) was created in 1993 specifically to help laboratories across the United States and internationally report HbAlc results that are traceable directly to the DCCT and UKPDS standards.[29] The NGSP has been remarkably successful in this effort.

Standardization of HbA1c measurement

In the 1980s and 1990s an important issue that concerned HbA1c measurement was the lack of standardization of the assay, and this meant that different analyzers could have widely differing reference intervals and yield varying results with patient samples.[30] As the DCCT and the UKPDS used the same method of analysis in their studies, this was felt to be a useful as the standard method for measurement of HbA1c. This method also has the added attraction that patients whose HbA1c has been tested by this method could have their HbA1c results compared directly with those of the subjects who participated in the two trials.[30,31] In order to develop this international harmonization, an extensive network of reference laboratories was established by the NGSP based in the USA. In the last decade this development has made great strides in making the HbA1c results reported from different laboratories much more comparable.[31,32]

However, the HbA1c results thus reported did not reflect the "true" HbA1c, but simply the best that 1980s technology could deliver when the DCCT study was conceived. In order to rectify this situation, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) developed a reference method for HbA1c. This method first defined reference material of purified HbA1c and HbA0, and then a highly specific reference method for their measurement.[33-35] HbA1c measurements using this technique are between 1.5% and 2% HbA1c lower than the NGSP results that relate to the DCCT.[36] This change has been resisted by some workers due to a fear of confusion occurring between the two sets of values. This could possibly result in clinicians either under-treating or over-treating patients because older targets are still being used.[36-41]

It was also suggested that rather than migrating to these IFCC values, the HbA1c could be expressed as a "mean plasma glucose equivalent", or, as it has recently been coined, "estimated average glucose" (eAG). There was much debate over whether to report HbA1c results in the traditional DCCT/UKPDS/NGSP units that most clinicians were familiar with or in the accuracy based IFCC units. The worldwide consensus that was arrived at on this issue[41] recommended the reporting of HbA1c in both the DCCT/UKPDS/NGSP units in % and the IFCC units in mmol/mol Hb. Additionally, results could also be reported as eAG if a subsequent study of the relationship between HbA1c and average glucose proved that this was feasible.[42]

HbA1c AS AN INDICATOR OF GLYCAEMIC CONTROL

Following a numerous studies there is a general acceptance of HbA1c as a useful tool to objectively assess the glycaemic control in patients with diabetes. In the DCCT, 1441 patients participated for an average of 6.5 years; 26,056 comparisons between HbA1c and a full 7- point profile were made. The study data were analyzed by linear regression analysis weighted by the number of observations per subject to correlate MPG and HbA1c. This analysis revealed the relationship of mean plasma glucose (mg/dL) = $(35.6 \times \text{HbA1c}) - 77.3$ with the $r = 0.82$. Since then, HbA1c has been used as the most accurate guide to clinicians and other healthcare workers when discussing glycaemic control with their patients.[43] The DCCT results developed a normogram based on their frequently sampled blood testing, finding a correlation coefficient of 0.80 between blood glucose and HbA1c.[44] On average, HbA1c of 6% corresponds to a mean plasma glucose of 135 mg/dL. For every increase in HbA1c of 1%, mean plasma glucose increases

Table 1: Correlation between HbA1c level and mean plasma glucose levels

HbA1c (%)	6	7	8	9	10	11	12
MPG (mg/dL)*	135	170	205	240	275	310	345

*Mean whole blood glucose results are 10%-15% lower. Most blood glucose meters are calibrated to read as plasma glucose
HbA1c=glycosylated haemoglobin; MPG=mean plasma glucose
Source: reference 45

by 35 mg/dL. Correlation between HbA1c and mean plasma glucose is shown in Table 1.[45]

HbA1c AND DIABETES COMPLICATIONS

The clinical utility of HbA1c as a tool to assess the risk of diabetes complications was proposed in the publication of the results of the DCCT[46] and also UKPDS[47] these studies set out to establish the effect of intensive (as compared with conventional) glycaemic control on the development of microvascular complications in type 1 and type 2 DM patients respectively.

Microvascular complications

The microvascular complications of DM comprise retinopathy, nephropathy and neuropathy. Patients with DM who develop these conditions constitute a large proportion of all subjects who develop blindness, renal failure and/or require limb amputation. The DCCT[46] found that when 1441 patients with type 1 DM were randomized to "intensive" rather than "conventional" treatment, their median HbA1c was 7.3% compared with 9.1% throughout the 6.5 years average follow-up period. The subsequent risk of developing retinopathy in the intensively treated group was reduced by 76%, the risk of developing proteinuria was reduced by 54%, and the risk of clinical neuropathy was reduced by 60%.[46] The risk of microvascular complications in both the patient groups rose exponentially as the HbA1c value increased.[48]

The publication of the UKPDS in 1998[47] confirmed that a relationship between HbA1c and microvascular complication risk existed.[47] There was still a 25% reduction in microvascular risk even though the difference in HbA1c between the intensive and conventional treatment groups was not as large as in the DCCT (HbA1c 7.0% Vs. 7.9% over 10 years). Later analysis has shown that when the two treatment groups are combined, a similar exponential relationship between rising HbA1c and rising microvascular risk could be discerned in UKPDS as in the DCCT.[50]

After the end of the DCCT, 96% of the patients in the original study agreed to continue to be followed up in a new study known as the Epidemiology of Diabetes Interventions and Complications study.[49] However, the patients were were no longer in two separate treatment groups. Following the outcomes of the DCCT, it was recommended that all patients follow an intensive treatment regime. It was therefore interesting that, out of a clinical trial scenario, the HbA1c of the previously intensively treated patients increased to an average of about 8%, while that of the conventionally treated group tightened up to a similar value.[51] Long-term follow-up of these patients has shown that the benefits of improved glycaemic control during the DCCT on the risk of microvascular complications are maintained in the long-term despite the convergence of glycaemia at the end of the original DCCT trial.[52-54] This observation that glycaemia from several years previously influences subsequent long-term complication risk has since been termed "metabolic memory"[55] and has reinforced the importance of good glycaemic control as soon as possible after the diagnosis of DM in order to avoid subsequent problems.

Macrovascular disease

Large vessel (macrovascular) disease remains the major cause of morbidity and mortality in patients with DM, with those having type 1 DM being at as high a risk as those with type 2 DM.[56,57] In type 1 DM, the DCCT found an excess of

macrovascular events in the conventional compared with the intensive group (40 versus 23), although this just failed to reach statistical significance (p=0.08).[58,59] Detailed analysis showed it was the mean HbA1c value during the DCCT that explained a large part of this beneficial effect on cardiovascular risk. In the UKPDS,[57,58] the event rate among the patients with type 2 DM was higher than in the DCCT, but the HbA1c separation between the two groups was less marked. Nevertheless, there was a suggestion of more myocardial infarctions among conventionally treated patients (p=0.052).[47] In a subsequent analysis, where the two treatment groups were combined, there was an overall relationship between rising HbA1c and increasing risk of myocardial infarction.[50]

HbA1c AS A DIAGNOSTIC TEST FOR DIABETES MELLITUS

DM is a chronic non communicable disease characterized by hyperglycaemia and is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several studies have reported that glycaemic control using fasting blood glucose, random blood glucose, oral glucose tolerance test (OGTT), HbA1c can reduce the development of and or progression to complications.[46]

Until recently, HbA1c was used as a key for monitoring glycaemic control in people with diabetes. More recently, it has been proposed as the preferred test for the diagnosis of DM[60,61] and it is being used in many areas to calculate an eAG concentration.[42] The problems with traditional blood glucose estimations include high intra-individual biological variability (4%-14%), preanalytical variability like the method of collection, storage (rate of fall of glucose in samples at room temperature 3-8 mg/dL/hour) and life-style measures like exercise and calorie restriction and difficulty in ensuring fasting state before blood glucose measurement.[62,63]

HbA1c overcomes many of these difficulties as it does not need a fasting state for sample collection or a glucose load and can be measured in a single sample collected at any time of the day. Additional advantages include analytical variability of less than 2% and information on glycaemic status over the past 2-3 months. Although HbA1c has recently been incorporated as a diagnostic test by the ADA, its validity needs to be established in Indian population. Until recently, HbA1c had not been recommended as a diagnostic or a screening tool because of several factors, such as, lack of standardization, low sensitivity and high cost.[64] However, following efforts at improving standardization of the HbA1c assay and the introduction of the new IFCC standards, HbA1c is now being considered for diagnostic and screening purposes.[64]

The advantages for HbA1c when compared with fasting glucose or 2h OGTT glucose are listed in Table 2.[60] ADA international expert committee proposed a HbA1c cut-off value of 6.5% as a diagnostic test for diabetes.[60] It is important to

Table 2: Comparison of various advantages and disadvantages of OGTT and HbA1c in the diagnosis of DM

HbA1c	Blood glucose measurement as a part of OGTT
Standardized and aligned to the DCCT, UKPDS	Less well standardized
Better index of overall glycaemic exposure and risk for long-term complications	Less well established as an index of overall glycaemic exposure and long-term complications
Substantially less biologic variability (<2 % day-to-day within person variability)	Higher likelihood of biologic variability (12%-15% day-to-day within person variability)
Substantially less preanalytic instability	Higher less preanalytic instability
No need for fasting or timed samples	Need for fasting or timed samples
Relatively unaffected by acute (e.g., stress or illness related) perturbations in glucose levels	Markedly affected by acute (e.g., stress or illness related) perturbations in glucose levels

OGTT = oral glucose tolerance test; DM = diabetes mellitus; HbA1c = glycosylated haemoglobin; DCCT = The Diabetes Control and Complications Trial; UKPDS = UK Prospective Diabetes Study

investigate whether these cut-off value for HbA1c apply to all populations worldwide. The normative distribution for HbA1c levels has been described in western populations in subjects with normal glucose tolerance (NGT) as well as impaired glucose tolerance (IGT).[65] However, there are few reports regarding normative HbA1c data from India which currently has the largest number of people with DM in the world. The comparison of studies defining a cut-off value in diagnosis of DM published from India and world is shown in Tables 3A and 3B.[66-74]

The use of a HbA1c level of 6.5% or higher to diagnose type 2 DM is now mainstream, with formal endorsements from three major U.S. medical associations in 2010 supporting an International Expert Committee's 2009 consensus recommendations. The International Expert Committee, with members appointed by the ADA, the European Association for the Study of Diabetes, and the International Diabetes Federation, set the ball rolling by publishing a consensus opinion in July 2009 to make HbA1c the preferred test for diagnosing type 2 DM.[60]

Table 3A: Comparison of studies published from India defining a cut-off value of HbA1c for the diagnosis of diabetes mellitus

Variable	Snehalatha et al[66]	Kumar et al[67]	Mohan et al[68]	Nair et al[69]
Year of publication	1998	2010	2010	2011
Place of study	Chennai,	Chandigarh	Chennai	New Delhi
Study design	Prospective	Prospective	Prospective	Prospective
No. of subjects studied	1261	1972	2188	525
Method of HbA1c estimation	Immunoturbidimetric method (Tina-Quant, Boehringer Mannheim, Germany)	HPLC-based ion exchange chromatography, Bio-Rad 10 (Bio-Rad, Hercules, CA, USA)	HPLC (Variant machine, Bio-Rad Laboratories, Hercules, CA, USA)	Turbidimetric immune inhibition method (SYNCHRON CX 5 using reagents from Beckman Coulter)
OGTT method	75 g anhydrous glucose	75 g anhydrous glucose	75 g anhydrous glucose	75 g anhydrous glucose
Blood sampling method	FPG and 2hPG; glucose oxidase/peroxidase method	FPG and 2hPG were estimated using a glucometer (Ultra 2; Johnson and Johnson, New Brunswick, NJ), which was validated*	FPG and 2hPG; glucose oxidase/ peroxidase method	FPG and 2hPG; glucose oxidase/peroxidase method
Method of defining HbA1c cut-off	ROC	ROC	ROC	ROC
Optimal HbA1c (%) cut-off for the diagnosis of DM	≥ 6	>6.5	>6.1†	≥5.8
Sensitivity (%)	88.5	65	88	75
Specificity (%)	62.5	88	87.9	75.5

* In every 10th case, peripheral venous plasma glucose was estimated by using the glucose oxidase method (Autoanlayzer 902; Hitachi, Tokyo, Japan). The correlation coefficients for FPG and 2hPG by glucometer and laboratory methods were 0.94 and 0.81

† for DM defined by FPG or 2hPG

FPG = fasting plasma glucose; 2hPG = 2-hour plasma glucose; ROC = receiver-operator characteristic curve; DM = diabetes mellitus; IGT = impaired glucose tolerance; IFG = impaired fasting glucose; HbA1c = glycosylated haemoglobin

Table 3B
Comparison of studies published from various parts of the world defining a cut-off value of HbA1c for the diagnosis of diabetes mellitus

Variable	Greci et al[70]	Esther et al[71]	Zemlin et al[72]	Kim et al[73]	Yun et al[74]
Year of publication	2003	2010	2011	2011	2012
Place of study	Derby, Connecticut, USA	Hoorn, Netherlands	Cape Town, South Africa	Cheonan, Korea	Nanjing, China
Study design	Prospective	Prospective	Prospective	Prospective	Prospective
No. of subjects studied	508	2753	946	224	497
Method of OGTT	75g anhydrous glucose	75g anhydrous glucose	75g anhydrous glucose	75 g anhydrous glucose*	75g anhydrous glucose
Method of HbA1c estimation	Boronate affinity binding assay (Abbott IMX Glycated Hemoglobin Assay, Abbott Laboratories, Abbott Park, IL, USA)	DCCT standardized reverse-phase cation exchange chromatography (HA 8160 analyzer; Menarini, Florence, Italy)	Turbidimetric inhibition immunoassay (Cobas 6000, Roche Diagnostics,USA)	HPLC (BioRad, Richmond, CA, USA)	Ion exchange HPLC (HLC-723G7 Analyzer, Tosoh Analyzer Manufacturers, China)
Method of defining HbA1c cut-off	ROC	ROC	ROC	ROC	ROC
Optimal HbA1c(%) cut-off	≥ 6.0	≥ 5.8	>6.5	>6.45	≥ 6.3
Sensitivity (%)	57	72	46	73.3	79.6
Specificity (%)	100	91	96	88.2	82.2

*performed for patients with FPG ≥ 100 mg/dL
DCCT = Diabetes Control and Complications Trial; FPG = fasting plasma glucose; ROC = receiver-operator characteristic curve; IGT = impaired glucose tolerance; IFG= impaired fasting glucose; HbA1c = glycosylated haemoglobin

The ADA translated the international consensus into clinical practice recommendations that were published in its annual update on standards of care in January 2010. The ADA backed away from calling HbA1c the preferred test, instead saying it's one of four diagnostic options, but acknowledged that it may become the most popular diagnostic test for type 2 DM.

OTHER APPLICATIONS

Recently, other applications of HbA1c have been described. HbA1c may predict incident cardiovascular events, even in individuals without DM,[75] HbA1c may be used as a predictor for fasting hyperglycaemia and metabolic syndrome. Increasing HbA1c was associated with increasing cardiovascular risk factors, so that HbA1c has been studied as a predictor of future development of cardiovascular risk.[75]

Despite the widespread use of HbA1c in monitoring diabetic patients, until now, this has not been used as the investigation of choice to diagnose DM. Despite the inclusion of HbA1c as an assay for defining diabetes, the American Association of Clinical Endocrinologists (AACE) has still not supported its use for the diagnosis of DM, though they mentioned HbA1c as an optional investigation. The reasons cited included difficulties in equating HbA1c with true glycaemia; variability between high and slow glycators, among others. Furthermore, a few added problems of HbA1c mandate caution for recommending HbA1c as a diagnostic test for DM. Data are not available as to how many laboratories in India are standardized for the HbA1c assay. Till there is reliable information regarding the availability of a standardized assay the test result cannot be relied upon. Many parts of India have a very high incidence of thalassaemia and other

haemoglobinopathies that limit the diagnostic utility of HbA1c in these geographical areas. The high prevalence of iron deficiency, especially in women in India, also renders HbA1c a poor choice as a diagnostic test. Sparse published data are available from India on slow and rapid glycators.

Considering all these points, more work is required before endorsing HbA1c as a diagnostic test for DM in India. As on today, HbA1c continues to be the best available option to monitor glycaemic control in patients receiving treatment for DM.

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