Review Article

Glycated haemoglobin; past, present, and future are we ready for the change

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Abstract

Glycated haemoglobin has been in use to monitor control of blood glucose in diabetic patients for about three decades. It provides an average blood glucose level during preceding 10 - 12 weeks. It is a very convenient blood test, can be done in any clinical setting regardless of prandial state. There were thirty different laboratory methods available to measure glycated haemoglobin with significant variability of results on same sample. IFCC developed a new reference method to measure the glycated haemoglobin, and the method is accepted world wide as only valid anchor for the measurement of HbA1c. In 2009 International expert committee recommended the use of HbA1c to diagnose diabetes with a threshold 6.5%. IFCC recommended the use of a new unit, i.e. mmol HbA1c/mol of total haemoglobin in place of percentage. Meanwhile a trial was conducted to find out relationship between average blood glucose and glycated haemoglobin, and a linear regression equation was developed to measure average blood glucose from HbA1c. Using the equation one can calculate average blood glucose from glycated haemoglobin in mmol/mol. This average blood glucose will be reported as "eAG" (estimated average glucose) and it will be used to monitor glucose control as eGFR (estimated glomerular filtration rate) is used to monitor renal function in chronic kidney disease patients. How easy or difficult would it be to abandon a term and a unit, in use for three decades and introducing a new unit (mmol/mol) and even a new term (eAG); only time will tell. Health professionals will need to familiarize with new term and units, they will also have to spend more time with their patients to educate them about new developments.

Introduction

Glycated haemoglobin is defined as haemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chains. This definition does not exclude haemoglobin that is additionally glycated at other sites on alpha or beta chains.[1]

HbA1c has been the most widely used and accepted test for monitoring the glycaemic control in individuals with diabetes. Once a haemoglobin molecule is glycated, it remains in the red blood cell for the rest of its life-span (120 days). As such, It provides information about the degree of long-term blood glucose control. The HbA1c level does not reflect an exact mean blood glucose; rather, it is weighted proportionally towards recent levels.[2] The formation of glycated Hb depends upon ambient glucose concentrations in which erythrocytes circulate as well as the duration of exposure. A whole blood sample for glycated Hb is sufficient regardless of prandial state and clinical setting.

Historical Perspective:

In 1955, researchers for the first time described, that adult haemoglobin contains heterogenous molecules. The significance of this finding was not explained till 1969 when Rahbar et al. described that unusual haemoglobin found in

Table-1

Take home message	
1	Elevated HbA1c even without a diagnosis of diabetes is independent risk factor for cardiovascular disease
2	HbA1c $\geq$ 6.5% is diagnostic for the Diabetes mellitus.
3	The HbA1c target is 7.0% in most treated patients with diabetes.
4	The ideal target of HbA1c is <6.0%, as long as it does not result in life threatening hypoglycaemia
5	The worldwide standardization of HbA1c was necessary given the significant mobility of today's world population.
6	The equivalent of the current HbA1c target 6.5% is 48 mmol/mol in the IFCC unit.
7	eAG would be used in diabetic patients as eGFR is used in patients with chronic kidney disease.

Table-2

Abbreviations Used	
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
ADA	American Diabetes Association
DCCT	Diabetes Control and Complication Trial
UKPDS	United Kingdom Prospective Diabetes Study
OGTT	Oral Glucose Tolerance Test
PCPG	Post Challenge Plasma Glucose
NGSP	National Glycohemoglobin Standardisation Programme
EASD	European Association for study of Diabetes
IDF	International Diabetes Federation
IFCC	International Federation of Clinical Chemistry.

A comparison of glycated Hb in different measuring units.

NGSP HbA1c (%)	IFCC HbA1c (mmol/mol)	eAG (mg/dL)	eAG (mmol/l)
4	20	68	3.8
5	31	97	5.4
6	42	126	7.0
7	53	154	8.6
8	64	183	10.2
9	75	212	11.8
10	86	240	13.4
11	97	269	14.9
12	108	298	16.5

diabetic patients was HbA1c, also noted a two-fold increase of HbA1c in diabetic patients.[3] By the mid 1970s, the nature of the chemical reaction had been explained. Glycation, is a spontaneous non-enzymatic reaction in which glucose binds covalently with haemoglobin at amino terminus of the β-globin chain.[4] In 1976, HbA1c was described as a useful mean for monitoring the glycaemic control in diabetic patients.[5] By the early 1980s, The HbA1c test was widely accepted in clinical practice.

Non-enzymatic Glycation verses Enzymatic Deglycation:

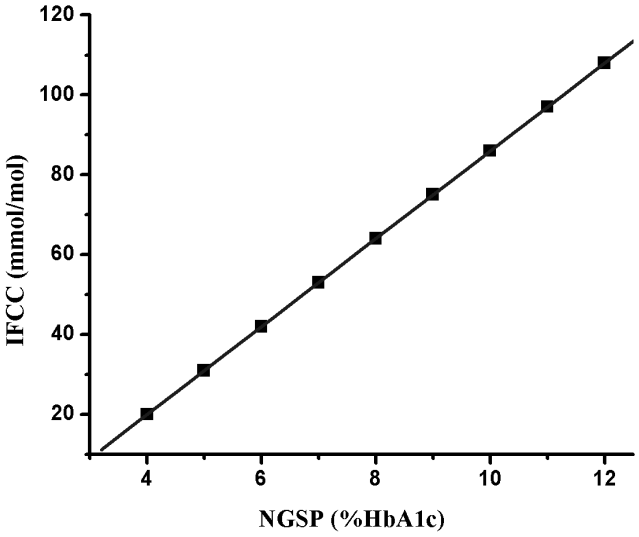
Most proteins (including haemoglobin) react with sugars to form covalent compounds without the involvement of enzymes. This chemical process is termed non-enzymatic glycation. The resulting accumulation of advanced glycation end products is associated with the progression of the complications of diabetes whereas enzymatic deglycation reverses the process of non-enzymatic glycation and generates free amino groups.[6] Enzymatic deglycation is a formidable defence system against non-enzymatic glycation in mammalian cells. This system operates using fructosamine-3-kinase (FN3K), phosphorylating fructoselysine residue on glycated proteins and thereby destabilizing the compound, ultimately causing the decomposition of the glycated proteins.[7,8] This process of enzymatic deglycation is overwhelmed by episodes of extreme hyperglycaemia in individuals with diabetes as non-enzymatic glycation continues unabated.[9] In the long run, it alters the stability of the protein structure, ultimately leading to cellular dysfunction.[10]

These Advanced Glycation End products (AGEs) directly and indirectly (via receptors) promote the development of cardiovascular disease.[11] They accumulate in different parts of the body and interact with receptors for advanced glycation end products (RAGE), induce oxidative stress, increase inflammation and enhance extracellular matrix deposition, thereby accelerating the process of endothelial dysfunction. Consequently, they result in accelerated plaque formation and ultimate atherosclerosis in diabetes.[11,12]

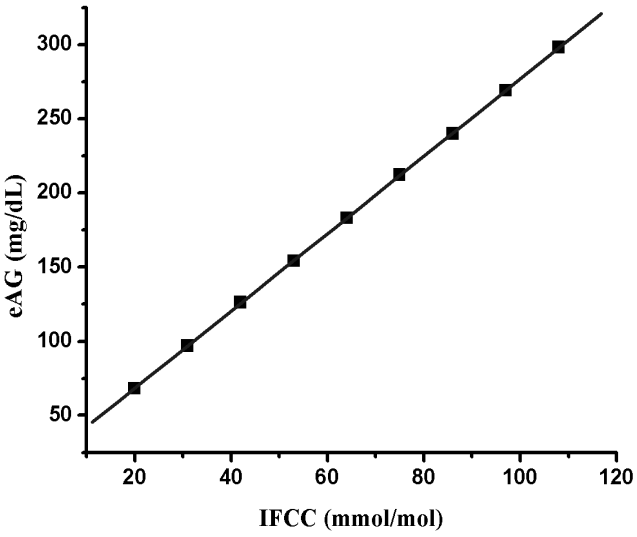
Glycated haemoglobin, intermediary compound is reversible but after some internal rearrangement of the compound, a stable HbA1c is formed.[13] Several glycation sites of the HbA molecule exist; N-terminal valine residue of the β-chain is the predominant glycation site, accounting for 60% of bound glucose. Of the three types of HbA1 namely, HbA1a, HbA1b, and HbA1c. HhA1c represents the most prevalent glycated species.

Clinical Use of HbA1c:

The world is facing an escalating epidemic of diabetes. More than 220 million people worldwide have been diagnosed with diabetes, although the actual number of people with diabetes is likely to be higher because of the insidious onset of Type 2 diabetes. Moreover, many people who have impaired glucose tolerance remain outside the diagnosed community of patients. The increasing life expectancy combined with the emergence of T2DM in children has resulted in phenomenal increase in diabetes related complications, becoming one of the major causes of disability and death worldwide. Type 2 diabetes accounts for 90% to 95% of all cases of diabetes. Furthermore, T2DM significantly increases the risk of heart disease and stroke; indeed, 50% of people with diabetes die of



Graph showing linear relationship between IFCC (mmol/mol) and NGSP (% HbA1c) units



Graph showing linear relationship between estimated average glucose and IFCC Units.

cardiovascular disease.[14]

In 2009 The International Expert Committee recommended the use of HbA1c to diagnose diabetes mellitus with a threshold ≥ 6.5%. However the diagnostic test should be standardized to Diabetes Control and Complication Trial (DCCT) reference assay or a method certified by National Glycohaemoglobin Standardisation Programme (NGSP).[15]

The use of HbA1c as a test went through nearly three decades of detailed scrutiny before being accepted as a diagnostic test for diabetes. Researchers had long been searching for test of glycaemia that could be used to screen and diagnose diabetes as well as monitor the chronic glycaemic control; such as test, may also be able to predict the onset of complications. Glycated haemoglobin acquires importance as a test for glycaemia because it has less intra-individual variation and is a better predictor of cardiovascular complications compared to fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT). In addition, it is used for glucose monitoring of diabetic patients.[16,17] In another study HbA1c and FPG showed continuous relationship with cardiovascular disease.[18]

While screening for diabetes, a relatively common condition, it is more important for the test to specifically identify patients than to be so sensitive as to diagnose many false positives, thereby making screening counter productive as this approach would put an extra burden on resources, especially in less privileged population of under developed countries.

Glycated haemoglobin has been extensively investigated by clinical trials, In 1979 P.J. Dunn et al[19] suggested that HbA1c is highly reproducible and responsive to changes in glucose tolerance; as such , it could be used to monitor the control of glycaemia. In 1991, Mulkerrin et al[20] reported very poor sensitivity (36%) and predictive value (44%) in their elderly sample (mean age 76 years old), thereby making HbA1c neither useful in screening nor beneficial for diagnosing diabetes. However, in acutely ill hospitalized patients HbA1c > 6% could reliably diagnose diabetes and level <5.2 % would reliably exclude diabetes.[21] In other words, the HbA1c cut-off of 6.5%, with very low sensitivity and very high specificity, could be used as a supportive marker for the diagnosis of diabetes.[22] Although HbA1c cut-off 6.5% for diagnosis is too high, it gives acceptable sensitivity and specificity rates at 44.6% and 99.6%, respectively.[23] A study from Australia suggested the use of a 7% cut-off rate for HbA1c when screening high risk populations.[24]

Other studies have asserted that simultaneous measurement of fasting blood glucose and HbA1c may be used to identify high risk patients at an early stage.[25,26] In 1994, McCane et al. recommended glycated Hb or fasting plasma glucose as an acceptable alternative for diagnosing diabetes instead of OGTT.[27] In one study most individuals with HbA1c at 6-7% had normal FPG but usually abnormal 2hour post challenge plasma glucose (PCPG); only 58% of patients with HbA1c in the 5% to 5.5% range had normal PCPG.[28] In a consensus statement: (2008) Society of Endocrinology recommended; 1.) HbA1c of 6.5-6.9% or greater, confirmed by FPG or OGTT should establish the diagnosis of diabetes, 2.) HbA1c ≥ 6.0% and impaired fasting glucose (≥ 100mg/dl) or random plasma glucose of 130-199mg/dl should lead to further diagnostic workup and closer follow-up.[29] By using these recommendations we may

identify a high proportion of individuals with undiagnosed diabetes, who would otherwise only be diagnosed once they developed end organ damage.

Standardization of Glycated Haemoglobin measurement; Why it is necessary?

Glycated Hb has been accepted as the gold standard measurement for the assessment of chronic hyperglycaemia for nearly three decades. There are thirty different laboratory methods available to measure glycated haemoglobin. Various analytical methods based on different assays principles, from ion-exchange chromatography to immunoassay and electrophoresis have been used to measure glycated haemoglobin. Such a lack of standardization resulted in wide variability within results (4.0% to 8.1%) on the same sample[30] making it difficult to compare patients results among laboratories. This disparity has always been a source of anxiety among health care providers. It becomes even more important in this age of heavy economical migration, when people travel long distances and take their native record with them. Therefore having same method and unit to measure HbA1c is need of the day.

To overcome this problem, in 1995 the International Federation of Clinical Chemistry (IFCC) took the lead in developing a uniform international standardization of HbA1c. For the calibration of the reference method, mixtures made of pure HbA1c and HbAo were developed. A laboratory network was also setup, which use two reference assays that combined reverse-phase high performance liquid chromatography (HPLC) with mass spectroscopy or capillary electrophoresis, using same mixture as calibrators. The IFCC then defined HbA1c as haemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta-chains.[1] This definition also covers Hb that is additionally glycated at any lysine residue in the β-chain. Prior to the IFCC's definition, HbA1c had been defined as a certain peak in an HPLC system, which obviously did not sound very scientific. Haemoglobin that is only glycated at a lysine site is not included in the measurement of HbA1c. Since the IFCC measurement is too specific, it only measures one molecular species of HbA1c: thus, non-HbA1c components are not included in final results. Consequently HbA1c values obtained by using IFCC method are 1.5 to 2 percentage points lower than the NGSP results traced to DCCT, as well as Swedish and Japanese designated comparison methods.[31]

Concerns were raised about the impact of this value change on patient care, which could result in less than desirable control of glycaemia in diabetic patients.[32] To overcome this problem a "master equation" was developed to formulize the relationship between the IFCC reference method and all three designated comparison methods (DCMs) namely, the National Glycohemoglobin Standardization program of US (NGSP), Japanese Diabetes Society/Japanese Society of Clinical Chemistry (JDS/JSCC), and Mono-S in Sweden.[33] The master equation allows for the conversion of the IFCC results to more customary HbA1c results, which could be traced to results from DCCT and United Kingdom Prospective Diabetes Study (UKPDS).

In 2004, the American Diabetes Association, European Association for the study of Diabetes, and International Diabetes Federation working group of the HbA1c assay was established to harmonize the reporting systems. It included members from the ADA, IDF, EASD, NGSP and IFCC. In 2007, the IFCC recommended that HbA1c results be expressed as mmol HbA1c/mol Hb instead of an HbA1c percentage. Patients using mmol/l or mg/dl for self-monitoring of day-to-day glucose control find it difficult to understand when their doctors discussed haemoglobin levels in percentages.

To eliminate confusion and streamline these discrepancies, a consensus statement[34] on the worldwide standardization of haemoglobin A1c measurement was adopted in May 2007 by the ADA, EASD, IDF and IFCC. It states that new IFCC reference system is the only valid anchor for implementing the standardization of the measurement of HbA1c. In addition, HbA1c results were to be reported worldwide in IFCC units (mmol glycated Hb / mol total Hb) and derived NGSP units (%), using the IFCC-NGSP master equation. Thus, the 25 to 42 (mmol/mol) range would indicate non-diabetics, as the similarly derived NGSP units of the non-diabetic range were 2.5 to 4.2% (HbA1c).

It was also resolved that if the ongoing "average plasma glucose study" was concluded successfully (i.e. confirmed the relationship between average blood glucose and HbA1c) then the A1c-Derived Average Glucose Equivalent would also be reported as an interpretation of HbA1c results.[34]

Relationship between Mean Blood Glucose and HbA1c:

Attempts to define a true relationship between average plasma glucose and HbA1c level have been made for some times, but studies had limited utility due to fewer measurements of glucose values and the limited number of participants involved. This method is error prone, with no night time samples collected, therefore, not a true representative of 24 hour glycaemia. Nathan et al. used continuous glucose monitoring, which measures interstitial glucose levels every 5 minutes, for 3 months in both non-diabetics and diabetics with relatively stable glycaemia. They reported a mathematical relationship between HbA1c and mean blood glucose, meaning HbA1c could be expressed in an equivalent mean glucose level (i.e., in the same units as

patients' self-monitoring units).[35] However this study is limited due to extremely small sample pool. A retrospective analysis of data from DCCT also identified a linear correlation between HbA1c and average blood glucose; however, the study population consisted of T1DM only, and DCCT was not designed to determine such a relationship.[36]

A New Term to Replace HbA1c:

The A1c-derived average glucose study[37] was conducted in 10 different locations in North America, Europe, and Africa. The two largest countries namely, India and China with huge diabetes population were left out, leaving it less representative. The study population comprised of 507 patients, 268 T1DM and 159 T2DM patients, and 80 non-diabetic subjects. The researchers sought to examine the relationship of average blood glucose with HbA1c across a wide range; (i.e. between HbA1c 5% to 13%). They collected approximately 2,700 blood glucose readings from each participant over 3 month period, the highest number of blood glucose readings per person to date in a single study. The goal of the study was to report glycated haemoglobin results not in the usual HbA1c percentage format but as A1c-derived averages in the same units used in self-monitoring, (i.e., mg/dl or mmol/l). The study concluded that the estimated average glucose (eAG) can now be calculated from HbA1c using a linear regression equation.

This eAG will now be used to monitor glycaemia in diabetic patients as the estimated glomerular filtration rate (eGFR), which is used to monitor chronic kidney disease, from the measurement of serum creatinine.

Conclusion

HbA1c levels are used to monitor glycaemic control throughout the world, and all major clinical trials including DCCT in T1DM and the United Kingdom prospective diabetes study (UKPDS) in T2DM have used it as a tool to monitor glycaemic control among the study population. Indeed, it did not happen over night; valuable time and resources have been spent to familiarize patients and health care providers with it. Yet after more than three decades of use, today only 25% of patients in a cross-sectional study were able to report their correct recent HbA1c, and 66% did not know their last HbA1c.[38]

Daily self blood testing, measured in mmol/L or mg/dl and HbA1c measurement in percentage are somewhat confusing. Given the narrow range of percentages, it is sometimes difficult for patients to comprehend the consequences of even a 1 percent increase or decline in HbA1c. Patients and their caretakers are used to the idea that the HbA1c level should be less than 7% in diabetic patients: a higher reading indicates that the glycaemic control is getting out of hand. Now the IFCC results will be provided in mmol HbA1c per mol haemoglobin. Keeping the NGSP results in percentages along with IFCC results will make the change less confusing.

With the introduction of the new term "estimated average glucose " (eAG). The eAG will be reported along with HbA1c results in the interim. Many physicians feel it would be easier to discuss eAG than haemoglobin in a diabetes out-patient. Others argue that the term eAG will only serve to confuse patients with diabetes: these experts also call for further research, especially among different ethnic groups and special circumstances (e.g., children and pregnant women). How soon all these controversies are resolved, culminating in this term being accepted in clinical use and entering into the lexicon, remains to be seen.

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