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Glycosylated Hemoglobin (HbA1c): An Indispensible Tool in the Management of Diabetes Mellitus

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The authenticity and use of HbA1c in the management of diabetes are established by many landmark trials and studies like the Diabetes Control and Complication Trial, (DCCT), UK Prospective Diabetes Study (UKPDS), Standardization of Glycated Hemoglobin testing, National Glycohemoglobin Standardization Programme, etc. These studies have developed the methods of HbA1c estimation, the reference range for identification of pre-diabetics and diabetics, and range at which micro and macro-vascular complications occur. If the pitfall of HbA1c is kept in mind, it is a proven tool in the management and follow-up of Diabetes Mellitus.

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Glycosylated Hemoglobin (HbA1c): An Indispensible Tool in the Management of Diabetes Mellitus

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I. Introduction

Diabetes mellitus was known to mankind since antiquity. Its mention is there as early as 1500 BC in Egyptian manuscripts, although the name diabetes mellitus was added later on. The term diabetes means to siphon (Greek) since the patient passes a large amount of urine and Mellitus means sweet like honey (Latin). [1]

The disease is characterized by elevated blood sugar and estimation of glycated Hb (HbA1c) remains one of the most important and indispensable investigations, besides estimation of blood sugar, fasting and postprandial. The blood glucose concentration depends upon the many factors like food intake, exercise, stress, etc, but the concentration of HbA1c in the blood reflects the average glucose over preceding 8-12 weeks. Thus estimation of HbA1c provides additional criteria to assess the control of Diabetes and is free from diurnal fluctuations which occur with blood glucose. The adaptation of HbA1c estimation has become an integral exercise in the management of diabetic patients because it offers Several advantages like patient need not be fasting, the sample can be collected at any time, it is stable and has got little biological variability. It also predicts the development of micro and macro vascular complications. [2], [3], [4]

a) The HbA1c

In normal adults, haemoglobin usually contains HbA (~97% of the total) (Table 1), HbA2 (~2.5%), and HbF (~0.5%).

HbA is made up of four polypeptide chains, two α- and two β- chains. The glycosylation is the non-enzymatic attachment of a sugar to the amino group of proteins. Analysis has revealed that HbA1c has a hexose attached covalently to the NH2-terminal valine residue of the β-chains of HbA. [5] The International Union of Pure and Applied Chemistry have defined HbA1c as the fraction of the β-chains of hemoglobin that has a stable hexose adduct on the NH2-terminal amino acid valine. [6]

b) The landmark trials/studies

1. The Diabetes Control and Complications Trial (DCCT) research group (1993) studied the effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. This study documented that, lowering blood glucose concentration as assessed by estimation of HbA1c resulted in the delayed onset and reduced rate of progression of microvascular complications in type 1 diabetics. It is a dominant predictor of retinopathy and cardiovascular complications. It unequivocally established the value of measuring HbA1c in patients of diabetes. [2]
2. UK Prospective Diabetes Study (UKPDS) in 1998. [3] This study was done to ensure that the results obtained by DCCT trials are comparable to with UKPDS trials. The estimation of HbA1c is done by ion-exchange, high performance liquid chromatography (HPLC) in DCCT Trials. Ten year follow-up demonstrated that the risk of myocardial infarction was significantly lower in patients who had lower HbA1c at the end of UKPDS trial. [7]
3. Standardization of glycated haemoglobin testing American Association for Clinical Chemistry (AACC)

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established a committee in 1993 to standardize Glycosylated haemoglobin testing. [8]

The NGSP (National Glycohemoglobin Standardization Programme) was created three years later to execute the protocol developed by AACC committee. The DCCT and UKPDS trials have established beyond doubt the direct relationship between HbA1c concentration and outcome risks in patients with diabetes. The concept is that all clinical laboratories should report an HbA1c value equivalent to that reported in the DCCT and UKPDS. [9] The goal of the NGSP is to standardize glycated hemoglobin test results to those of the DCCT and UKPDS.

4. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in 1995. The primary objective of this committee was to develop a true reference method for HbA1c. [10] Instead of standardizing to a comparison method, the primary objective of the IFCC committee was to develop a true reference method for HbA1c.The NGSP and IFCC networks have complementary roles in the HbA1c standardization process. The two networks work together to form a solid basis for the establishment of a reliable and accurate HbA1c measurement platform.
5. The master equation a linear relationship can be derived between HbA1c results of IFCC reference method and NGSP network. [11] The calculated regression equation is:-

$$NGSP=0.09148(IFCC)+2.152.$$

This equation is called "master equation" and permits conversion between the two sets of values. But, the HbA1c values measured by the IFCC method are significantly lower than the NGSP values, and moreover, the difference is not constant.

The three preeminent clinical diabetic organizations, the American Diabetic Association (ADA), the European Association for the Study of Diabetes (EASD), and the International Diabetes Federation (IDF), attempted to resolve the dispute by agreeing to consider reporting HbA1c with estimated average glucose (ear). [12], [13] a linear relationship was established between HbA1c and mean blood glucose. [14]

c) Reporting of HbA1c

Another significant development was how to report HbA1c.In 2007 IFCC adapted reporting by International System of Units (SI units) rather than the percentage. This type of reporting avoids the confusion of using percentage (as the unit), which is having different reference interval. Thus the IFCC values are now expressed as millimoles of HbA1c per mole of HbA1c. [15], [16]

In 2004, a working group was constituted with representatives from ADA, ESAD, and IDF with a view to harmonizing HbA1c reporting. This group was termed as ADA/ESAD/IDF working group of the HbA1c assay. The consensus statement published in 2007 reiterates that the HbA1c should be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%) using IFCC-NGSP master equation. [17]

Reporting of HbA1c

HbA1c estimation is recommended by International guidelines as a preferred measure when evaluating the overall control of diabetes and patient's risk for complications. [18], [19] HbA1c is recognized as a reliable marker for the overall glucose exposure and its direct consequences. In setting a target level of HbA1c in an individual patient, due consideration should be given to patient's health, the risk of hypoglycemia, co-morbidities, and his/her specific health issues. The IDF (International Diabetes Federation) and the American College of Endocrinology (ACE) recommend HbA1c values below 6.5% while ADA (American Diabetes Association) recommends control below 7.0% for most patients. [20] Thus the estimation of HbA1c has become a key measure for diagnosing, screening and monitoring diabetes, and is an indispensable tool.

i. *Criteria for Identification of Prediabetic*

A Prediabetic is defined as, a person, having impaired fasting glucose and impaired glucose tolerance, based on 2-hour OGTT (oral glucose tolerance test) and FPG (Fasting Plasma Glucose). The patients having HbA1c values 6.00% to 6.49% are considered at high risk for developing diabetes as advised by ADA and WHO. [21], [22], [23]

Table 1: Criteria for identification of Prediabetic. [23]

Measurement	ADA, 2015 criteria	WHO, 2006/2011 criteria
Haemoglobin A1c	5.7%–6.4% (39–46 mmol/mol)	6.0%–6.5% (42 mmol/mol)
Fasting plasma glucose	100-125 mg/dL 5.6-6.9 mmol/L	110-125 mg/dL 6.1-6.9 mmol/L
2 hour post-prandiol plasma glucose	140-199 mg/dL 7.8-11.0 mmol/L	140-200 mg/dL 7.8-11.1 mmol/L

ADA = American Diabetes Association; WHO = World Health Organization

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ii. *Criteria for diagnosis of Diabetes*

The 1997 Expert Committee on the Diagnosis and Classification of Diabetes Mellitus encouraged that that it is based on the glycaemic level at which microvascular complications develops. [24] Evaluating HbA1c in 0.5% increments, investigators found that the incidence of diabetic retinopathy rose above baseline at HbA1c of 6.5%, the now accepted diagnostic value.The committee established that there is increased risk of diabetic retinopathy at fasting plasma glucose level greater than or equal to 126mg/dl (7.0mmol/L). It was done to define a cut-off point of HbA1c for diagnostic purposes. In a DETECT-2 trial, the investigators found microvascular complications above 6.5% hbA1c. [25] Diabetes can be diagnosed by using venous plasma sample for HbA1c and fasting plasma glucose, 2-hour oral glucose tolerance test (OGTT) ads to the diagnostic accuracy. OGTT also identifies the patients with impaired glucose tolerance; this along with impaired fasting glucose is a marker for impaired beta cell function with future progression to frank diabetes.

Random plasma glucose >200mg/dL (11.1 mmol/L) and symptoms of hyperglycemia like polyurea, polydipsia, blurred vision and weight loss also confirm the diagnosis (of Diabetes Mellitus). At any given time fulfillment of any two of the three criteria clinches to the diagnosis. [23]

Table 2: Criteria for the diagnosis of Diabetes. [23]

Measurement	ADA 2015 diagnostic value
Haemoglobin A1c	> 6.5 %(48 mmol/mol)
Fasting plasma glucose	> 126 mg/dL (7.0 mmol/L)
2-hour postprandial plasma Glucose.	> 200mg/dL (11.1 mmol/L)

ADA= American Diabetic association

Table 3: HbA1c and corresponding estimated average glucose. [26]

HbA1c %	Mean plasma glucose (mg/dL)	IFCC Units (mmol/mol)
6	126	42
6.5	141	48
7	154	53
7.5	169	59
8	183	64
8.5	198	69
9	212	75
9.5	226	80
10	240	86
11	269	97
12	298	108

IFCC= International Federation of Clinical Chemistry and Laboratory Medicine

d) Pitfalls in HbA1c Measurements

i. *Falsely lowered HbA1c levels*

The condition that shortens the life-span of RBCs or causes a rapid turnover of it shortens the exposure of cells to as follows:

Acute or chronic blood loss due to any causes, hemolytic anemia, spherocytosis, splenomegaly, Hemoglobinopathies and associated anemia, chronic malaria, glucose-6-phosphate-dehydrogenase (G-6-Pd) deficiency, sickle cell anemia etc, since they change turnover of RBCs. Erythrocyte survival is also shortened in chronic hyperglycemic states. This decreased survival may underestimate the severity of hyperglycemia at higher HbA1c levels. [27], [28]

The end-stage renal disease generally has falsely low HbA1c levels. It is due to chronic anemia and reduced red cell survival. So in end-stage renal disease, the clinician should take into consideration this fact while evaluating glycaemic status. [29], [30]

Pregnancy: A1C may not be a true reflection of glycaemia during pregnancy primarily because of both the decreased life span of the red blood cell from about 120 days to about 90 days as well as increased erythropoietin production. So, it is advised not to use the criteria of HbA1c levels for the diagnosis of gestational diabetes. [31]

Miscellaneous: the vitamin supplements like Vitamin-E, Ribaverin, and interferon Alfa are associated with falsely lowered HbA1c levels.

ii. *Falsely elevated HbA1c levels*

Any condition that prolongs the lifespan of red blood cells or decreases its turnover may cause a false elevation of HbA1c levels. These conditions include Iron deficiency anemia, vitamin B-12, and folate deficiency anemia.[32] Uremia, hypertriglyceridemia (concentration > 1750mg/dL), severe hyperbilirubinemia can also show falsely elevated HbA1c levels [33-34] So in the presence

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one has to be cautious in its interpretation. Salicylates, opiates and lead poisoning may be responsible for the false elevation of HbA1c levels. [35] While making changes in therapy based on elevated HbA1c levels these conditions must be taken into consideration to avoid, inappropriate therapeutic regimen and hypoglycemia.

In situations where the HbA1c results do not give a true picture due to false elevation or false low results, it is desirable to use alternative indices. These include fructosamine, glycated albumin, 1, 5-Anhdroglucitol (1, 5-AG), and continuous glucose monitoring (CGM).

II. Conclusion

The measurement of plasma glucose is the most reliable mean to diagnose diabetes mellitus. HbA1c measurement remains the gold standard to monitor glycaemic control and also an important diagnostic criterion. The results should be interpreted keeping in mind that, the other clinical conditions and co-morbidities, may give falsely elevated or lowered levels of hbA1c. In such situations, the alternative measure of glycaemic control should be considered like fructosamine, glycated albumin, 1, 5-AG or continuous glucose monitoring. The importance of the estimation of HbA1c is that it is validated as a predictor of diabetes-related outcomes. The diagnostic HbA1c cut-off point of 6.5% is associated with retinopathy prevalence and is the diagnostic thresholds for Fasting plasma glucose and 2-hr post-prandial-glucose. The HbA1c range of 5.7-6.4% in addition to impaired fasting glucose (IFG), and IGT (impaired glucose tolerance) have been included in the "categories of increased risk for diabetes".

The study of HbA1c has answered a very basic question of glycaemic control after which complications are prone to occur. It has got certain inherent limitation, despite that estimation of HbA1c remains the gold standard in the management of Diabetes mellitus.

The key learning points

1. An HbA1c level ≥ 6.5% is the cut-off point, used for the diagnosis of diabetes mellitus.
2. The values of HbA1c in the range of 5.7% to 6.4%.are included in the "categories of increased risk for diabetes".
3. HbA1c is formed by the glycosylation of Haemoglobin, so anything which interferes with a lifespan of RBCs or process of glycosylation may give a false result.
4. The use of Hba1c levels for monitoring of diabetes control should take into consideration the other co-morbidities.
5. The combination of FGT and HbA1c significantly enhances the diagnostic accuracy of these tests.
6. The estimation of HbA1c has unique potential of assessing the retrospective glycaemic control as well as predicting a risk for retinopathies and cardiovascular risks.
7. Thus the HbA1c test continues as an important diagnostic and prognostic tool, for the better patient care and successful clinical outcome.

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