CREST

A Division of Coral Clinical Systems

TECHNICAL SERIES

Glycated Hemoglobin (GHb)

The marker for retrospective glycemic control

Tulip Group

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Foreword

Coral Cinical Systems is a part of the innovative **TULIP** Group of companies based at Goa, India.

Coral manufactures reagents and kits for general pathological analysis. **Crest Biosystems,** a division of **Coral,** manufactures liquid stable reagents and kits for Clinical Biochemistry. The product range includes most enzymes, substrates and the largest range of kits for the estimation of metals & ions.

The group's commitment in building products of international standards, through indigenous R&D has accorded the company virtual leadership in most product segments in the Indian marketplace. Its state of-art manufacturing facility conforms to the strictest FDA (India) and GMP regulations. In its efforts to build world-class Quality products, the group has recently received the ISO 9001(2000) certification from TUV. It is this commitment to Quality, which has given the group international acclaim. The products are now exported to over 45 countries globally with an ever-increasing user base. With decades of experience in *in-vitro* diagnostics (IVD), **Tulip** Group has created a strong knowledge base. **Tulip** Group believes that in the knowledge-based society of the 21st century, regular upgradation of knowledge is essential not only for better diagnosis and patient care, but also to improve the overall quality of life.

Publishing of Technical Series is one such initiative to make available to the Laboratory professionals and clinicians updated knowledge that is vital for them to set trends in their day-to-day practice.

INTRODUCTION

Worldwide projections suggest that more than **220 million people** would be affected by Diabetes Mellitus **(DM)** by the year 2010. **In the U.S.A.** alone, health care costs due to Diabetes Mellitus would be approximately **14% of the healthcare budget**.

For reasons of genetics & lifestyles, **Indians form the world's largest diabetic population.** Today, **India has 25 million diabetic patients, more than any other country** & the number is expected to rise to 35 million by 2010 & to 57 million by 2025.

It is reported that **in urban India 12% of the adult population suffers from Diabetes**, compared to 6% in the U.S.A. & the U.K. Strangely though, **in the rural** Indian population (comprising about 70% of the total population) , **a mere 2-3% suffer from Diabetes.**

WHAT IS DIABETES MELLITUS (DM)?

Diabetes Mellitus is a group of metabolic disorders, connected by raised / elevated levels of plasma / serum, glucose concentration, either when fasting, or after ingestion of carbohydrates, often accompanied by the presence of glucose in urine, arising from a disturbance of glucose metabolism.

During Diabetes, glucose is under utilized resulting in hyperglycemia. This under utilization of blood glucose is caused by a relative or absolute deficiency of insulin. It occurs with different degrees of severity & in its severest form causes keto-acidosis, coma & untimely death if not managed appropriately.

CLASSIFICATION OF DIABETES MELLITUS

The World Health Organization **(WHO**) classification of DM is as follows:

TYPE 1 Diabetes Mellitus (TYPE-1 DM)

Also known as Insulin dependent diabetes mellitus (IDDM) & / or Juvenile Onset diabetes mellitus, this is characterized by the autoimmune / viral / chemical toxins, induced destruction of the beta cells of the pancreas. The patients require insulin to avoid keto-acidosis. This type of diabetics constitutes roughly 10 - 20 % of the total diabetic population

TYPE 2 Diabetes Mellitus (TYPE-2 DM)

Also known as non insulin dependent diabetes mellitus (NIDDM), adult onset diabetes, it comprises between 80-90% of the diabetic population. In this case , the pancreas retain some beta cell capacity, resulting in insulin levels that vary,but in all cases are less than that required to maintain glucose homeostasis..

TYPE-2 DM is frequently accompanied by target organ insulin resistance, which results in a decreased responsiveness to both exogenous & endogenous insulin, which in some cases can be due to a decreased number of insulin receptors on the target cells.

The occurrence of TYPE-2 DM, is almost completely determined by the genetic factors. There appears to be very little, if at all, involvement of viruses or autoimmune antibodies. The metabolic alterations observed are milder than those for the TYPE-1 DM.

Other forms of DM:

Genetic defects of insulin action

Diseases of the exocrine pancreas

LONG TERM COMPLICATIONS OF UNCONTROLLED DM

Elevated Fasting plasma glucose (FPG) is considered to be a basic indicator of DM. Once the condition is established, regular & appropriate control / monitoring / assessment of the patient condition is critical to decrease morbidity & mortality due to DM, with its associated debilitating complications.

Vascular complications in DM may be classified as micro-vascular predominantly, affecting the retina, kidney & the nerves and macro-vascular which, predominantly affect the coronary artery, cerebrovascular and peripheral arterial circulation.

Uncontrolled DM, leads to **acute complications** such as:

Hypoglycemia, occurring in more than 90% cases of TYPE-1 DM.

Keto-acidosis develops where there is an absolute or relative insulin deficiency.

Hyperosmolar non-acidotic diabetes, of which there is no satisfactory evidence differentiating it from diabetic keto-acidosis.

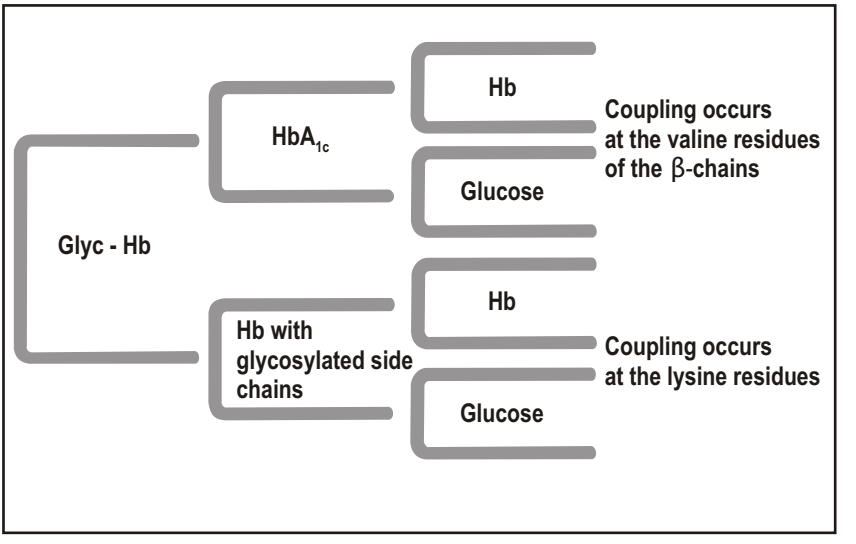
Somogyi effect, which is a unique combination of hypoglycemia during night with a rebound hyperglycemia in the morning. This condition is more common in TYPE-1 DM and more so in children.

Dawn phenomenon, where there is a early morning rise in blood glucose levels with no hypoglycemic episodes in the night.

Infections, where a variety of factors predisposes the diabetic patient to an increased incidence, or increased severity of infections.

The test is simple, provides reproducible results & quantitative help in adjusting the therapeutic approach.

The ultimate goal in Diabetes management is to provide the patient with skills & ways to manage their chronic illness as well to provide motivation to sustain the efforts to do so. GHb measurements are helpful in achieving both these goals.



The "Elements" - Glucose & Hemoglobin:

Glucose

Considered to be nature's fuel, glucose is a ready source of energy, since its carbon atoms are easily burnt (oxidized) to form carbon dioxide, releasing energy in the process. The importance of glucose can be well understood by the fact that an average human adult has roughly 5-6 grams (1 teaspoon) of glucose circulating in the blood, which will supply the body's energy needs for roughly 15 minutes. Thereafter the levels must be replenished from internal reserves or external sources.

Structurally glucose exists in both the ring (pyranose) & the linear forms. While it is relatively inactive in the ring form, the free aldehyde linear form can react at a number of sites, especially the amino groups of proteins.

The erythrocytes (RBC) being freely permeable to glucose (not insulin dependent), an elevation of the plasma glucose level leads to a simultaneous elevation of the glucose level within the RBC's.

Hemoglobin

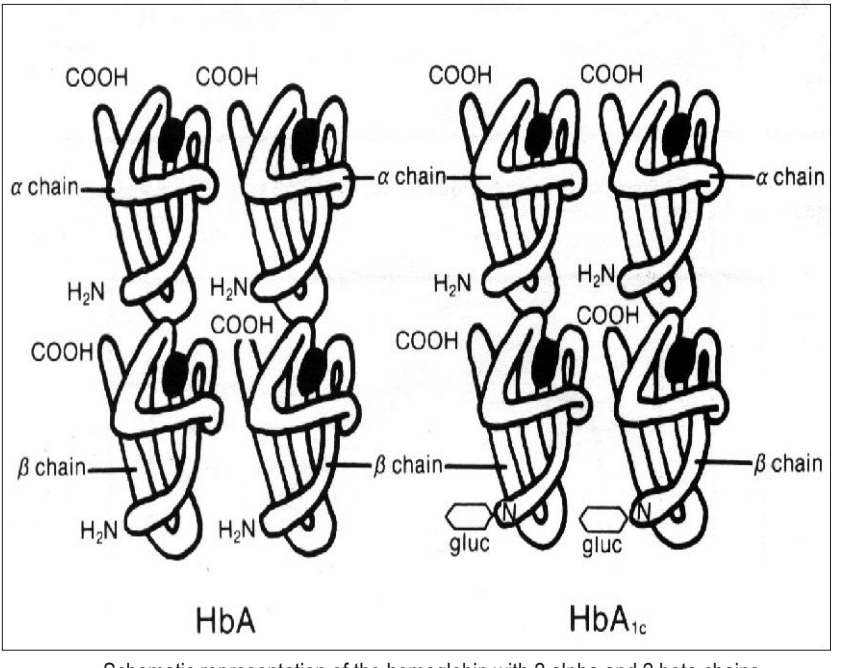
Circulating within the erythrocytes (RBC-average life span of 120 days), hemoglobin (Hb) with a molecular weight of 64,000 daltons, is the primary oxygen carrying vehicle (protein) in the human body.

Structurally the Hb molecule consists of two α & two non α chains, of which, the following variations are found in the healthy adult hemoglobin :

Fetal Hb	-	$2\alpha + 2\gamma$; < 1%	(designated HbF)
Adult Hb	-	$2\alpha + 2\beta$; ⩾ 97%	(designated **HbA**)
		$2\alpha + 2\delta$; < 2.5%	(designated HbA2)

The 2 α & 2 β chains of normal **adult hemoglobin (HbA)** are structurally similar, with each chain containing a heme molecule & a series of amino acids (141 & 146 for alpha & beta chain respectively).

Due to the unique molecular structure, the hemoglobin molecule presents a number of reactive sites on the N-terminal valine as well as several lysine residues on both the alpha & the beta chains.



Schematic representation of the hemoglobin with 2 alpha and 2 beta chains

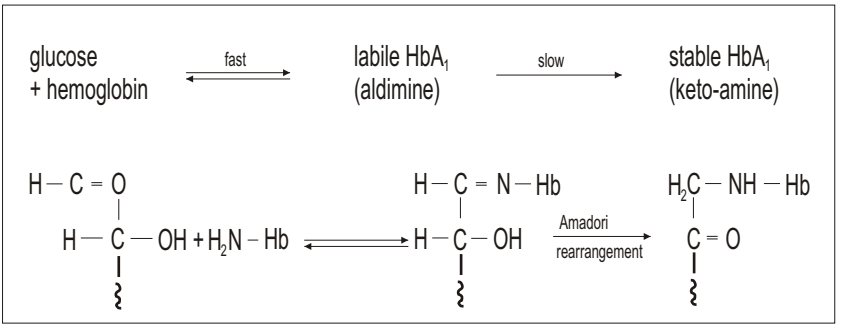
The Process of Glycation.

The term GLYCATION is suggested & currently accepted by the IFCC / IUPAC for all such nonenzymatic reactions that link a sugar to a protein or peptide. The product of glycation is a glycoprotein, or, in the special case of the reaction with hemoglobin, glycohemoglobin, or Glycated hemoglobin's **& as such the term Glycated hemoglobin is used throughout in this text.**

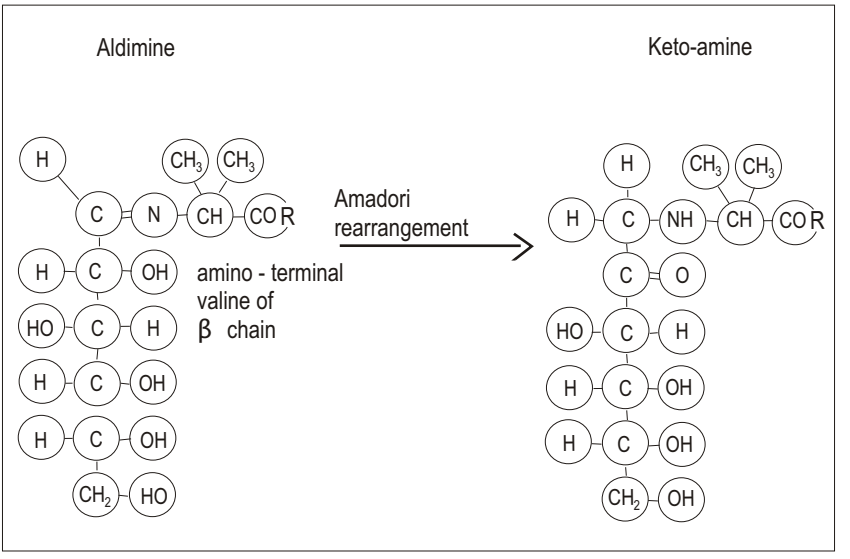
In a normal person glucose circulates in the blood. The erythrocytes are freely permeable to glucose and the concentration in the cell is approximately the same as in the plasma. When the glucose levels are elevated in plasma, they are proportionately elevated in the erythrocytes.

When the glucose is in its free aldehyde form, it can react with the hemoglobin molecule present in the erythrocytes. The free aldehyde group of the glucose attaches to one or both of the N-terminal valine of the β chain or the free amino group of lysine of the hemoglobin molecule to form a Schiff's base or an aldimine. This process is relatively fast and reversible. It depends on the glucose concentration in the erythrocytes. As the glucose molecule has a hydroxyl group at carbon-2, it can undergo a shift in the double bond to carbon-2 via what is known as an Amadori rearrangement to form a keto -amine

This is a slow process and once formed is relatively irreversible. The stable ketoamine form, with the glucose molecule attached to the hemoglobin remains for the life span of the erythrocyte. This process is called **Glycation**.



The Glycated Hemoglobin (GHb) is formed continuously by the adduction of glucose by covalent bonding to the amino terminal valine of the hemoglobin chain progressively and irreversibly over a period of time and is stable till the life of the erythrocyte. This process is slow, non-enzymatic and is dependent on the average blood glucose concentration over a period of time.



Reaction of glucose with hemoglobin at the amino terminal valine of hemoglobin to form labile and stable adducts.

TYPES OF GLYCATED HEMOGLOBIN

Of the normal Hemoglobin, HbA, the glycated hemoglobin is also referred to as Hemoglobin A1 (HbA1) and the non glycated portion as HbAo. HbA1 comprises of several fractions depending on sugar, which has glycated the hemoglobin.

HbA1a	
HbA1a1	Glycation with Fructose 1, 6 diphosphate
HbA1a2	Glycation with Glucose 6 phosphate
HbA1b	Glycation with an unknown reaction partner
HbA1c	Glycation with D glucose
I HbA1c	denotes the labile HbA1c, or the aldimine fraction
S HbA1c	denotes the stable HbA1c, or the ketoamine fraction

As the Glycated hemoglobin remains in the erythrocytes till their life span of around 120 days and its formation is unaffected by diet, insulin or exercise on the day of testing, it reflects the averaged blood glucose levels over the last several weeks. Thus, GHb reflects on the long term control and status of the carbohydrate metabolism of the past 6-8 weeks.

Methods for the determination of glycated hemoglobin.

These can be broadly classified into the following categories:

Methods based on charge difference
Methods based on chemical reactivity
Methods based on structural differences
Methods based on immunochemical techniques

1. Methods based on charge differences

These are mainly of two types:

A. Cation exchange chromatography
B. Electrophoresis

A. Cation exchange Chromatography

These are mainly of three types:

i. Columns
ii. High Performance Liquid Chromatography (HPLC)
iii. Resin slurry

Glycated Hemoglobin is less positively charged at a neutral pH than HbAo and binds less strongly to a negatively charged resin. Hence it is eluted first and then followed by the HbAo to separate the same. Usually this is done in three ways:

- Columns / micro columns: the hemolysate is applied to a column filled with resin and the elute is collected. The less positively charged HbA1a, HbA1b and HbA1c are eluted before the HbAo. Then their percent to the total Hemoglobin can be determined. The results are affected by temperature, pH, ionic strength and column size.

  HPLC: These systems use a column and elute the different fractions, HbA1a, HbA1b, HbA1c and HbAo successively by using different buffers having different ionic strength and pH. These methods show excellent precision and permit rapid separation of HbA1c. The cost of equipment and its running can only be done by some large laboratories or research institutions.

  Resin slurry method: Here the HbAo is bound to the resin and the HbA1 remains in the supernatant buffer. The resin bound with the HbAo is separated using a resin separator and the absorbance of the HbA1 is measured. The absorbance of the total Hemoglobin is measured separately. The results are calculated by taking the ratio of the sample against a control or standard.

B. Electrophoretic methods

Glycated hemoglobin can also be measured by electrophoresis, or isoelectric focusing. The various hemoglobin components are separated according to their charge differences. These methods are time consuming, require specialized equipment for electrophoresis, densitometer, etc. In the separation of the hemoglobin there is interference by aldimine and HbF.

2. Methods based on chemical reactivity

These are colorimetric methods using thiobarbituric acid. The sugar attached to the hemoglobin is converted to hydroxymethylfurfural (HMF). The process requires treatment with heat and acid and is quantified by the color formed with thiobarbituric acid. The method is time consuming and as the free glucose of the sample interferes it has to be first removed before testing. Results are expressed as nanomoles of HMF / mg of Hb or fructose equivalent /mg of Hb. Colorimetric analysis gives rise to falsely elevated values unless specimens are handled appropriately. The presence of free glucose with hemoglobin also adversely affects the assay.

3. Methods based on structural differences

In these methods the determination of GHb is done by affinity chromatography. The test is normally done with micro columns which have a resin/gel charged with M-aminophenylboronic acid. The sugar portion of the glycosylated hemoglobin has an affinity for the boronic acid and binds with the same. The HbAo is first eluted using a buffer and then the HbA1 is eluted with a buffer containing sorbitol which has a greater affinity to the boronic acid and displaces the HbA1, which then elutes out. Some of these methods also have buffers which only displace out the HbA1c. These methods are sensitive to gel properties such as ligand concentration.

4. Methods based on Immunochemical techniques

These are recent methods being used on fully automatic systems based on the immunoturbidimetric principles. A hemolysate of the sample is added to the reagent containing latex particles coated with an antibody to HbA1c and the amount of turbidity formed due to the binding of the antibody and the antigen (HbA1c) is measured colorimetrically at around 550nm. In some tests a further reagent is added which contains other antibodies or an agglutinator which binds all the hemoglobin's and further rise in turbidity is measured. The ratio of HbA1c to total hemoglobin is then calculated.

Glycated hemoglobin determination is useful in monitoring the long term status of the carbohydrate metabolism or the glucose level in the body. It helps the clinician in monitoring the status of the patient especially in the maintenance programme of improved glycemic control. The GHb test can also be used as a diagnostic assay for the detection of TYPE-1 DM, as normally these patients are hyperglycemic and their GHb levels are usually high. (Though it must be kept in mind that the **GTT is a more definitive test.)**

CLINICAL IMPORTANCE OF THE GHb DETERMINATION

Evaluation of widely varying blood sugar values

Evaluation of glucose values in doubtful cases:

- checks performed by the patient are unsatisfactory
- unreliable patient non compliance
- diagnosis of diabetics without any previous clinical symptoms
- short term illness of the patient

Support & control of the checks performed by the patient

- encouragement towards achieving the glycemic targets
- it is a parameter for the therapeutic monitoring for the patient
- The measurement excludes incorrect interpretations etc.
- it is a long term retrospective control of metabolic measurement independent of the actual blood sugar concentration.

The only factors on which the test is dependent are

the circulating levels of blood glucose &

the life of the erythrocytes

CLINICAL INTERPRETATION OF GHb TEST:

* Subjects with normal metabolism - 5 - 8% GHb

* Suspect range - 8 - 10%"

* Unsatisfactory control - > 10%"

Relation Of GHb Values to that of the mean blood glucose

Degree of Glucose Control	GHbA1	HbA1c	MBG/dl
Normal (non diabetic)	⩽ 7 %	< 6 %	<114
Near (Borderline) Normoglycemic but targets	7 - 8 %	6 - 7 %	114 - 147
Diabetics, but, in good control	8 - 9 %	7 - 8 %	147 - 180
Actions suggested	9 - 11 %	8 - 9 %	180 - 214
Diabetics & not at all in control	> 11 %	> 9 %	> 214

The MBG (mean blood glucose) values above are arrived at by using the formula used in the study done by **Nathan et al.**

MBG in mg/dl = 33.3 x HbA1c value - 86

It is however to be noted that the relationship in the above formula is linear in the range of HbA1c values falling between 6.5 - 13%.

Significance of GHb measurement

A single fasting blood glucose measurement only gives an indication of the patients immediate past (last 2-4 hours at most) condition & is dependent on many factors. It may not necessarily represent the true status of the blood glucose regulation.

In contrast the level of glycated hemoglobin (GHb) is directly related to the average plasma glucose concentration and the life span of the RBC's and thus hemoglobin in the circulation and is independent of any other factor.

Conclusive evidence towards the reduction of chronic risks of unmonitored DM, through regular GHb estimation

The Projects

Study	DM type	No/Subjects	Mean Age	Period of Assessment
DCCT	TYPE-1	1441	27 years	10 years
UKPDS	TYPE-2	3867	54 years	5 years

Objectives

The above two multicenter randomized clinical trials were designed to compare the benefits / risk reduction of long term chronic complications of DM, with intensive therapy, compared to those given the conventional management of TYPE-1 & TYPE-2 DM, respectively.

RESULTS

Estimating GHb values regularly & maintaining them in the normoglycemic range in those receiving the intensive therapy showed dramatic results as under:

Relative risk reduction for chronic diabetic complications

RISK	DCCT	UKPDS
Retinopathy	reduced by 63%	reduced by 21%
Proteinuria	reduced by 54%	reduced by 34%
Neuropathy	reduced by 60%	reduced by 40%
Coronary Artery Disease	reduced by 41%	reduced by 16%

Frequency (need based assessment) of GHb testing:	
Nature of Patient	**Recommended GHb testing frequency**
Controlled diabetic patient	once every three months
Diabetic pregnant woman	once a month
Patient whose diabetes regimen has been altered to improve control or in whom evidence is present that intercurrent events may have altered a previously satisfactory level of control	once every 1 - 2 months
For uncontrolled TYPE-1 / TYPE 2 diabetics	more than four times a year

(Source; American Association of Clinical Endocrinologists guidelines for the management of DM)

Factors that interfere with GHb Test results

Hemoglobin Variants and Derivatives

Genetic variants (e.g. HbS trait, HbC trait) and chemically modified derivatives of hemoglobin (e.g. carbamylated Hb in patients with renal failure, acetylated Hb in patients taking large amounts of aspirin) can affect the accuracy of GHb measurements. The effects vary depending on the specific Hb variant or derivative and the specific GHb method. When selecting an assay method, laboratories should take into consideration characteristics of the patient population served, (i.e., high prevalence of hemoglobinopathies or renal failure).

Shortened Erythrocyte Survival

Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia) will falsely lower GHb test results regardless of the assay method used.

GHb results from patients with HbSS, HbCC, and HbSC must be interpreted with caution given the pathological processes, including anemia, increased red cell turnover, transfusion requirements, that adversely impact GHb as a marker of long-term glycemic control. Alternative forms of testing such as glycated serum protein (fructosamine) should be considered for these patients.

In patients with renal failure, GHb has been reported to be elevated despite the fact that RBC survival rate may be decreased in patients with uremia. Carbamylation

reaction appears to account for some of the change in the physical properties of the hemoglobin in uremia. Cyanate , a breakdown product of urea, can react with amino groups in an irreversible fashion & thus change chromatographic properties.

It is now known that in a diabetic population, there are a proportion of people who appear to glycate hemoglobin at a faster or slower rate than most others. What it means is that **two individuals with the same degree of glucose tolerance might well have their glycated hemoglobin values differing by 2%, which is substantial.** Originally, it was thought that differences in inter-individual tissue glycation was the reason, but recent studies have data that establishes that **the high glycators seem to have red blood cells that survive longer than the low glycators. The changes in the GHb levels appear to be related to the red blood cell life rather than glycemia or glycation rates.**

Alcohol consumption

Acetaldehyde, the major breakdown product of alcohol, has been shown to change the chromatographic pattern of hemoglobin. Thus **patients consuming large amounts of alcohol may have falsely elevated levels of GHb.**

Other factors

Vitamins C and E are reported to falsely lower test results, possibly by inhibiting glycation of hemoglobin; vitamin C may increase values with some assays. Iron-deficiency anemia is reported to increase test results. Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, chronic ingestion of salicylates, and opiate addiction are reported to interfere with some assay methods, falsely elevating the results.

Other Glycated Proteins

Serum proteins with shorter half lives (2-3 weeks) such as **albumin** also become heavily glycated in the presence of persistently elevated glucose levels. Thus total glycated serum proteins (GSPs) & glycated serum albumin (GSA) can provide an estimation of **glycemic control over the 14 - 20 days.**

Fructosamine Test

Fructosamine is the generic name for plasma protein ketoamines. The name refers to the structure of the ketoamine rearrangement product formed by the interaction of glucose with the epsilon amino group on the lysine residues of the albumin. Circulating half-life for albumin being roughly 20 days, the concentration of glycated albumin reflects glucose control over a period of 2 - 3 weeks. Although the assay can be automated, gives good precision, there is till date a lack of consensus on its clinical utility due to:

Apparent lack of specificity for glycated proteins
Lack of standardization among laboratories
Difficulty in calibrating the assay
Interferences by urates & hyperlipidaemia

As per the recommendations of the National Medicare Programme for Diabetes (USA), Glycated protein testing may be used in place of glycated hemoglobin in the management of diabetic patients who have abnormalities of erythrocytes such as hemolytic anemia or hemoglobinopathies. In such cases it is conceivable that a patient will have both a GHb and GSP ordered on the same day. However this should be limited to the initial assay of GHb, with subsequent exclusive use of GSP. Moreover, clinicians should be aware of two important points concerning the use of GSP's & GSA as measures of long-term glycemic control:

These tests assess the degree of glycemic control over a period of 2 weeks as opposed to 2 - 3 months with GHb.
Neither test has been correlated with the development of long-term complications from diabetes mellitus as was shown with GHb in the DCCT and with the UKPDS.

GUIDELINES FOR LONG TERM GLYCEMIC CONTROL

In light of the substantial evidence now available in support of tighter glycemic control it is today possible to optimize reduction of microvascular & macrovascular complications of diabetes. Surprisingly though, despite the widespread use of self monitoring of blood glucose, **patients do not know their diabetes status & goals. It has also been found and established that in a vast majority of populations, diabetes was present long before diagnosis was made.**

As a consequence of the enormity & gravity of the situation, the following issues were raised, discussed & clarified, as being the **global guidelines of long term Glycemic control:**

1) what is the goal of diabetes management?
2) to what extent glycemic control attains that goal?
3) what factors should be used to assess glycemic control?
4) what are the guidelines for the attainment for glycemic control?
5) what further recommendations are needed regarding glycemic control & reduction of complications of diabetes?

The goal of diabetes management is the prevention of acute & chronic complications of diabetes mellitus. Traditional complications of DM are viewed as microvascular complications of diabetes including retinopathy, nephropathy, & neuropathy.

Reductions in hyperglycemia significantly decrease the microvascular complications of diabetes. Prevention of eye and kidney damage by intensive diabetes management in the DCCT & Kumamoto study reduced the incidence of these complications by as much as 50 - 70 %. All trials demonstrated a 30 - 35 % reduction in microvascular complications per 1% absolute reduction of glycated hemoglobin.

It was the consensus that glycemic control be assessed by the periodic measurement of glycated hemoglobin.

Fasting glycemia (FPG) can measure the glycemic burden only at a single point in time & does not accurately reflect the overall glycemic control. Of course, with the advent of self monitoring technology, assessments of fasting & pre-prandial plasma glucose levels have evolved into an important element of day to day decision making in the routine management of diabetes.

Post-prandial hyperglycemia is a key component of the total glycemic burden in patient with diabetes; it is an important contributor to the Glycated Hemoglobin levels, which can be viewed as reflecting the summation of both preprandial & postprandial glycemia. Thus for maximum reduction of Glycated Hemoglobin levels, assessment of both pre-prandial & post-prandial glucose levels are necessary as part of diabetes management.

Studies from the DCCT & UKPDS analysis of data have implicated the association of high Glycated Hemoglobin levels with the development of complications of diabetes, especially atherosclerosis. It has thus been recommended that **Glycated Hemoglobin be universally adopted as the primary method of assessment of glycemic control.**

It is also **recommended that the Glycated Hemoglobin estimations be performed at least twice a year in patients who are a target**. Assessments should be made quarterly, or more frequently in patients who are above target, who are undergoing a

change in therapy, or both. Patients with diabetes should be identified as early as possible in their illness. Current recommendations are towards targeted screening for populations at high risk for the development of diabetes. Risk factors include:

Family history of diabetes
Cardiovascular disease
Overweight
Sedentary lifestyle
Latin/Hispanic/African/ Asian ethnicity
Previously identified impaired GTT, or IFG
Hypertension
Increased levels of triglycerides, low concentrations of HDLC
History of gestational diabetes
Delivery of a baby weighing more than 4 kilograms
Polycystic ovary syndromes.

The high frequency of complications at the time of diagnosis of diabetes makes earlier diagnosis imperative. It is also seen that within certain ethnic groups the onset of diabetes is at a much younger age within the general population. Thus, it is recommended that targeted case finding in high risk persons 30 years of age or older is undertaken.

CONCLUSION

In conclusion, it may be argued that even though glycemic targets set are perhaps "unrealistic" & not quite achievable by most, it however contradicts the clinical judgement that **patients who achieve ANY reduction in their levels of Glycated Hemoglobin, significantly decrease the risk for complications of diabetes regardless of their ability to achieve the specific glycemic targets** recommended by the various panels.

Therefore, it is now accepted that setting of clinical standards should reflect the best estimate of maximal benefit that may accrue from that care & should not depend on the ease or convenience of attaining such targets for either patients or clinicians.

Most importantly it is now sincerely believed that establishing glycemic targets would assist & encourage patients & clinicians strive towards achieving normoglycemia; that in itself would result in improved health, augmented longevity & an enhanced quality of life.

These, after all, are the goals of diabetes management.

GLOSSARY

Diabetes Insipidus
Condition leading to polyuria & polydipsia caused by the deficiency of Anti Diuretic Hormone (ADH), usually due to trauma or tumour involving posterior pituitary.

Gestational Diabetes Mellitus (GDM)
Defined as any degree of glucose intolerance with onset or first recognition of pregnancy. In majority of the cases of GDM, the glucose levels will return to normal levels after delivery.GDM complicates only around 4% of all pregnancy cases.

Impaired Glucose Tolerance (IGT)
Identifies a group of individuals whose response to the ingestion of a glucose load lies between normal & diabetic responses. A portion of these subjects eventually becomes diabetic, but some remain stable & few revert back to normal.

Glycation
The term currently accepted by the IFCC & IUPAC for any non enzymatic attachment of a carbohydrate moiety to a protein molecule.

HbA
The major form of adult hemoglobin comprising between 95-97% of the total hemoglobin.

HbAo
The major component of normal adult hemoglobin, which is the labile non Glycated portion , separated out during the GHb assay.

HbA1
More negatively charged, post- translationally modified, Glycated form of HBA,
Comprising of the following chromatographically distinct stable fractions:
HBA1a / HBA1a2 / HBA1b / HBA1c

HbA1c
The IFCC defines HBA1c as the stable ketoamine form (adduct) of gluose to N-terminal valine of the β chain of the hemoglobin.

Variant
The normal genetic composition of the Hb tetratmer is $\alpha 2\beta 2$ & to a lesser extent

(3-4%) $\alpha 2\delta 2$ (HBA2),and $\alpha 2$ 2 (HbF) <1%. Any hemoglobin types other than these are called variants. Majority of variants arise from point mutations of either α , β, γ or the δ Hb chains. Till date more than 850 variants have been identified.

DCCT

Diabetes control & complications trial ; a landmark multicenter evaluation of Glycemic control carried out over a period of ten years in the United States.

UKPDS

United Kingdom Prospective Diabetes Study; another landmark evaluation of long term Glycemic control, carried out in U.K. over a period of five years.

DN

Characterized by marked hyaline thickening of the glomerular basement membrane as a result of deposition of proteins or glycoproteins from plasma, Diabetic Nephropathy, refers to damage of the glomerulus (filtering apparatus of the nephron) & capillaries associated with the nephron, leading to a reduction in the filtering capability of the kidneys & presenting a clinical picture of marked Proteinuria (> 3.5gm/day),red blood cell cast,oedema, & hypertension. DN develops in 30-40% cases of TYPE-1, & 10 - 20% of cases with TYPE-2 DM, and is the single, most common cause of end stage renal disease (ESRD). There is a correlation between diffuse diabetic glomerulosclerosis & deterioration of renal function determined by increased Diastolic blood pressure, proteinuria, blood urea, & creatinine with corresponding decrease in serum albumin, urea & creatinine clearances.

CAD

The pathogenesis of Coronary Artery Diseases (CAD) in DM is not entirely clear & conventional risk factors such as smoking, obesity, blood pressure, serum lipids fail to fully explain this. Important features in the pathogenesis of CAD appear to include vascular endothelial injury, platelet adhesion and activation, fibrin deposition, cellular proliferation & LDL cholesterol accumulation. Therefore disturbances of haemostasis due to DM leading to accelerated fibrin formation (hypercoagubality), & delayed fibrin removal (impaired fibrinolysis) may contribute to CAD. Hyperactive platelets, hypercoagubility & impaired fibrinolysis also promote thrombosis formation at the site of a ruptured atherosclerotic lesion leading to the final occlusion event in the progression of CAD. .

Chronic complications resulting from long term sequelae of unmonitored DM are:
Retinopathy
Neuropathy
Angiopathy
Diabetic Nephropathy (DN)
Thrombosis
Atherosclerosis / Coronary Artery Disease (CAD).
Diabetic Nephropathy (DN) & Coronary Artery Diseases (CAD), account for more than two thirds of all deaths among diabetics.

LABORATORY DIAGNOSIS OF DM

The three routine laboratory diagnostic tests used for establishing DM in a patient are:

Random plasma / serum glucose test **(RPG)**
Fasting plasma / serum glucose test **(FPG)**
Oral glucose tolerance test **(OGTT)**

Diagnostic algorithm for DM

Test	Range	Diagnosis
RPG	⩾ 200 mg/dl	DM
	⩽ 99 mg/dl	DM excluded
	101 - 198 mg/dl	FPG suggested
FPG	⩾ 126 mg/dl	DM
	⩽ 110 mg/dl	DM excluded, but suggested OGTT to diagnose **Impaired glucose Tolerance (IGT)** in Absence of IFG.
	110 - 125 mg/dl	**Impaired Fasting Glucose (IFG)** but suggested OGTT
OGTT (2 hour glucose)	⩾ 200 mg/dl	DM
	⩽ 140 mg/dl	No IGT
	140 - 199 mg/dl	IGT

Adapted from the WHO & International Diabetes Federation guidelines. All glucose concentrations refer to venous plasma.

In the absence of classical symptoms (thirst, polyurea, unexplained weight loss) RPG is the recommended first test & at least two results on different days are essential. If results are discordant, 2-hour OGTT result takes precedence, but one should consider re-testing after an interval.

UTILITY OF THE CLINICAL TESTS IN ESTABLISHING & MONITORING DM

While all the tests (RPG / FPG / OGTT) measuring the mean plasma glucose, are currently used for the diagnosis of the condition , **the tests themselves are subject to inaccuracies & variations** due to various factors such as :

short term illness of the patient

stress

non-compliance by the patient, such as irregular & uncontrolled eating habits otherwise, but a strict regimen of diet & medication prior to the test being performed, leading to a "misleading" glycemic status.

All the above tests reflect the body's metabolic state at the time of the test, at best giving an indication to the immediate glycemic status of the body (2-4 hours prior to sampling , at the most) & hence are not suitable as a marker for long term monitoring of the condition (DM).

As is evident, not only is the establishment of the condition (DM) important, **it is equally if not more important to monitor the Glycemic status of a patient,** once the condition has been established & confirmed.

GLYCATED HEMOGLOBIN (glycosylated hemoglobin - GHb)

The extensively researched marker, currently in use for the long term monitoring of the Glycemic status among diabetics, is the Glycated hemoglobin test.

Whereas all the other tests measuring glucose indicates only the immediate Glycemic status , **Glycated hemoglobin (GHb) is the only test that gives the measure of mean blood glucose (MBG) "round the clock" for the last 6 - 8 weeks.**

Given the importance of long term monitoring & benefits of appropriately managing DM, the testing, for levels of Glycated hemoglobin (GHb- as being the only such long term marker),assumes a critical position in the overall management of DM. GHb values provide an extremely useful clinical tool in quantitating glucose levels over the previous 6 - 8 weeks.

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Notes:

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