Glycated Haemoglobin- Recent Developments and Review on Non-Glycemic Variables

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Abstract

Glycated hemoglobin (HbA1c) is the current tool for monitoring glycemic control once a diagnosis of diabetes is established. Its role in the diagnosis of diabetes has only recently come to attention. In the past, many international organizations have discussed the role of HbA1c in the diagnosis of diabetes and rejected this application as appropriately DCCT-aligned assays were not used or available globally. Considering the high biological variability, the dynamics of glucose, as well as the limitations of blood glucose monitoring technology, at that time, the possibility of obtaining an integrated average glycemia value by the measurement of a single biomarker elicited immense interest and provided a powerful tool in both diabetes research and clinical management. HbA1c testing was soon facilitated by the development of a new analytical methodology that was suitable for use in clinical laboratories. However, a consensus statement in 2007 on assays used to report HbA1c has now further strengthened the case for a change in the diagnosis of diabetes. Using HbA1c as a screening or diagnostic tool has some logistical advantages over traditional glucose testing (either oral glucose tolerance test [OGTT] or fasting plasma glucose [FPG]). Patients can present for a relatively quick test in a non-fasted state at any point of the day, allowing more scope for opportunistic screening. HbA1c assay readings are less prone to recent influences of physical or emotional stress and provide an indication of longer term glycemic control spanning the last 2–3 months. Owing to such logistical advantages there are calls for HbA1c to become the preferred diagnostic tool over glucose tests. Performing the HbA1c test regularly allows the assessment of glycemic control and verification of the efficacy of medication treatment and of education for self-care. It is estimated that 33% to 49% of people with DM2 cannot achieve adequate goals for glucose, blood pressure, or lipid profile control and only 14% reach normal parameters in these measurements.

Keywords- *Diabetes Mellitus, Glycated hemoglobin, Hyperglycemia, Blood Glucose.*

Background

Diabetes mellitus refers to a group of metabolic disorder that shares the phenotype of hyperglycemia characterized by insulin resistance initially, impaired insulin secretion, insulin deficiency, increase glucose production and decreased glucose utilization and the complications arising from this disease is the major cause of death worldwide. The cells of the body cannot metabolize carbohydrate due to relative or complete lack of insulin and body breaks its own protein, fat, glycogen resulting in hyperglycemia.[1-3]

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Glycated Hemoglobin

Glycated hemoglobin (HbA1c) was first described by Rahbar *et al*. in 1969.[4] Hemoglobin (Hb) is a tetramer

formed of two alpha and two beta globin chains. On exposure to high levels of blood glucose, hemoglobin gets non-enzymatically glycated at different sites in the molecule.[5] Glycation is the non-enzymatic addition of sugar residue to the amino groups of proteins. Glycated Hb also known as HbA1c , is used as a guideline to check the status of patients glycemic status for preceding 3 months or it represents value for glucose preceding 8 to 12 weeks and provides criteria for assessing glucose control, Glycated Hb values are free of day to day glucose fluctuations and unaffected by recent exercise or food ingestion.[6,7]

HbA1c of 6.5% is recommended as the cut point for the diagnosis of diabetes.[8] Human Adult Hb consists of HbA (97% of the total, it is made up of 4 polypeptide chains, 2α and 2β), HbA_2 (2.5% , made up of 2α and 2δ) and HbF (0.5%, and made up of 2α and 2γ). Several minor hemoglobin's are identified in chromatographic analysis of HbA_1, namely, HbA_{1a}, HbA_{1b} and $HbA_{1c,}$ which are referred as HbA_1, fast hemoglobins, glycohemoglobins, or glycated hemoglobins .[6,9]

HbA1c is formed by combination of aldehyde group of glucose and hexoses non-enzymatically which binds with amino terminal of the β-chain of Hb, to form an unstable Schiff base (aldimine, pre-HbA1c) before undergoing an Amadori rearrangement to form a more stable ketoamine, HbA1c and this process is known as glycation of Hb occurs in life span of 120 days. So this characteristic of Hb biomarker is used to monitoring the average blood glucose levels.[9,4] So the measurement of HbA1c is used for checking blood sugar control in pre-diabetic patients and monitoring sugar levels in patients with elevated HbA1c.[10]

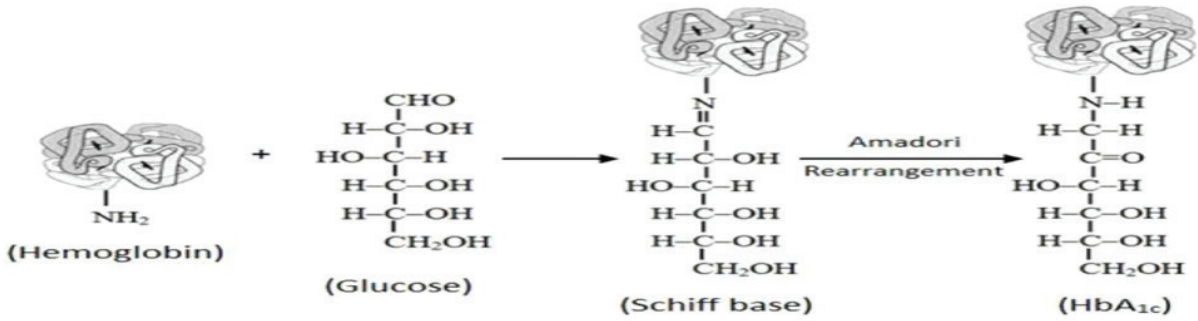

Figure 1. Formation of HbA1c[9]

Non-glycemic Variables affecting HbA1c

Non glycemic variables affecting HbA1c are age, ethnicity, gender, erythrocyte turnover, anaemia, pregnancy, Haemoglobin variants, thyroid disease, liver disease, HIV and Kidney diseases. It has long been recognized that hemoglobin variants interfere with HbA1c synthesis and measurement, and this interference depends on the nature of the congenital disorder afflicting hemoglobin synthesis and the analytical method that is used to measure HbA1c. [11] Thalassemia traits, HbS, HbC, HbE and HbF are among the most abundant hemoglobin-related interferences.[12]Additionally, other posttranslational modifications of hemoglobin such as carbamylation by uremic toxins in end-stage renal disease may significantly interfere with some HbA1c assays.[13] It should be noted that the majority of interferences have been mitigated by improvements of analytical methodologies, and the remaining interferences have been depicted and rigorously scrutinized.[14]

Age was found to be associated with a gradual increase of HbA1c levels in non diabetic individuals independently of sex and level of glycemia, indicating that age-specific reference intervals/clinical cut-off points may improve the clinical accuracy of this test in both the diagnosis and management of diabetes.[15] There are ethnic differences in HbA1c values even when glycemia levels are the same; a recent meta-analysis revealed that Caucasians have slightly lower HbA1c values in comparison to persons of other ethnic groups .[16] While the clinical relevance of this finding needs

to be further investigated, the understanding of the molecular mechanisms behind this observed between-race variability in HbA1c may improve its clinical applicability. Nonglycemic factors affecting HbA1c levels include erythropoiesis, hemoglobin synthesis and conditions influencing red blood cell survival. Deficiency anemias generally elicit falsely increased HbA1c levels due to the increased levels of aged erythrocytes that are found in patients with this disease, whereas falsely decreased HbA1c levels can be observed in hemolytic anemias of any cause.[17] Nonhematological conditions influencing HbA1c values include pregnancy, chronic renal failure and certain medications.[11] Variability in the normal erythrocyte lifespan is another significant confounder of HbA1c accuracy. Malka et al[18] recently proposed a mechanistic mathematical model integrating hemoglobin glycation and red blood cell kinetics that provided a personalized insight into average glucose levels and reduced the occurrence of diagnostic errors due to a misinterpretation of average glycemia (as reflected by HbA1c) by more than 50%. The applicability and clinical utility of the proposed model have yet to be determined.

The glycation extent of haemoglobin (glycated haemoglobin A1c) is an indicator of plasma glucose levels over the ~100 days prior to a venipuncture, and the laboratory assays for its quantification are steadily changing.[19,20] The remainder of glycoproteins are collectively termed fructosamines [21], and most of these are glycated albumin. Investigations into both HbA1c and fructosamine values are appropriate for clinical use. [22,23]The difference between the measured HbA1c value and the HbA1c value predicted from fructosamine values is termed the glycation gap. It has a broad distribution in patients with nephropathy (from −3.0% to 5.5%) .[24] Glycation gap values oscillating within >−0.5 and 0.5 was considered metabolically unfit. [25,26]Glycation gap evaluations currently comply with the reference change value criteria for glycaemic control, as documented by their stability in the follow-up of patients with type 2 diabetes.[27] To date, the glycation gap ranges in healthy controls are unknown. It has been suggested that glycation gap is not associated with chronic kidney disease in nondiabetic individuals .[28]

GLYCATED HB IN THE DIAGNOSIS OF DIABETES

Diabetes mellitus is a chronic non communicable disease characterized by hyperglycaemia and associated with long-term complications. DM management requires an accurate evaluation of glycaemic control. The traditional blood glucose estimations give results that are influenced by biological variability, variability due to pre-analytical factors (such as blood collection method and blood sex hormones in glucose homeostasis as well as to different attitudes and behaviours related to diabetes care.

In 1985 WHO report mentioned the utility of HbA1c in diabetes. By 2010 ADA and major expert committee and association across the globe recommended HbA1c for the diagnosis of type 2 diabetes mellitus.[4] In 2010 ADA-organized international expert committee and recommended adoption of HbA1c for the diagnosis of diabetes at a cut off 6.5%[9] clinical significance of HbA1c in T1DM was given by the Diabetes Control and Complications Trial (DCCT) and significance on T2DM was given by United Kingdom Prospective Diabetes Study. Measurement of HbA1C and blood glucose levels are used in routine management of patients with T1DM and T2DM. [10]

HbA1c measurement is recommended for checking blood sugar control in pre-diabetic and monitoring blood sugar control in patients with elevated level.[10]

Table 1: Factors that influence HbA1c and its measurement[10]

1.Erythropoiesis Increased HbA1c: Vitamin B12 deficiency, decreased erythropoiesis, Iron. Decreased HbA1c: Administration of erythropoietin, iron, vitamin B12, reticulocytosis and chronic liver disease.
2. Altered Haemoglobin Chemical or Genetic alterations in haemoglobin: Haemoglobinopathies, HbF, methaemoglobin, may increase or decrease HbA1c

Cont... **Table 1: Factors that influence HbA1c and its measurement[10]**

3.Glycation Increased HbA1c: Alcoholism, chronic renal failure, decreased intraerythrocyte pH. Decreased HbA1c: Aspirin, Vitamin C and E, certain intra-erythrocyte pH. Variable HbA1c: Genetic determinants.
4.Erythrocyte destruction Increased HbA1c: Splenectomy, increased erythrocyte life span, . Decreased HbA1c: Haemoglobinopathies, splenomegaly, rheumatoid arthritis or drug such as antiretrovirals, ribavirin and dapsone, decreased RBCs life span
5.Assays Increased HbA1c: Hyperbilirubinaemia, carbamylated haemoglobin, alcoholism, large doses of aspirin, chronic opiate use. Variable HbA1c: Haemoglobinopathies. Decreased HbA1c : Hypertriglyceridaemia.

Pathophysiology of Hyperglycemia

1. Non-enzymatic Glycation of proteins, mainly haemoglobin, low density lipoproteins, collagen and tubulin in pheripheral nerves leads to accumulation of advanced glycosylated end products causing injury by stimulating pro-inflammatory factors (complement and cytokines).

2. Polyol pathway: There is accumulation of sorbitol and fructose in the glucose metabolism by increased intracellular aldose reductase which leads to cell proliferation, changes in vascular permeability and capillary structure by stimulation of protein kinase C and transforming growth factor beta.

3. Abnormal microvascular blood flow impairs nutrient and oxygen supply. Microvascular occlusion due to vasoconstrictors (endothelins and thrombogenesis) and leads to endothelial damage.

4. Other factors formation of Reactive oxygen species and stimulation of growth factor TGF-β and VEGF. These growth factors are released from ischaemic tissues and causes proliferation of endothelial cells.[29]

METHODS OF MEASURING HbA1c[30]

Method of Testing	**Procedure**	**Advantage**	**Disadvantage**
Chromatography based HPLC assay	Assay uses an HPLC instrument and ion exchange or affinity column to separate HbA1c molecules from another hemoglobin molecules.	HbA1c overestimation leads to aggressive glucose management, resulting in more frequent hypoglycaemic episodes.	a. Altering the normal process of glycation of HbA to A1C. b. Causing an abnormal peak on chromatography, making estimation of A1C unreliable. c. Making the red blood cell more prone to hemolysis, thereby decreasing the time for glycosylation to occur and producing a falsely low A1C result.

Cont... **METHODS OF MEASURING HbA1c[30]**

Boronate affinity	Glucose binds to m-aminophenylboronic acid	Minimal interference from hemoglobinopathies, HbF, and carbamylated Hb	Measures not only glycation of N-terminal valine on β chain, but also β chains glycated at other sites and glycated α chains
Antibody based immunoassay	Uses a specific antibody (usually monoclonal) to the glucose and the first 5 to 10 amino acids of the β-chain. This antibody is latex coated. The agglutinator reacts with the antibody to give a scattering of light and an increase in absorbance. From this the amount of HbA1c is calculated, and the total hemoglobin can be determined by measuring at or near the Soret absorption band of hemoglobin (410 - 420nm) or by Drabkins method (oxidation and conversion to cyanmethemoglobin) at about 540nm, or using the alkali hematin assay.	Reduces the scattering of light and the absorbance.	a. Time required to complete the analysis. b. Technical skills required for handling. c. High price of reagents.
Enzyme based enzymatic assay	Lysed blood samples are subjected to proteolytic digestion. Glycated valines are released and serve as substrate for fructosyl valine oxidase. The produced hydrogen peroxide is measured using a horseradish peroxidase-catalyzed reaction with a chromogen	a. Enzymatic assay proved to be a robust and reliable method for HbA1c measurement suitable for routine practice in clinical chemistry laboratories. b. The assay is designed to report %HbA1c values directly without need for a separate measurement of total hemoglobin and is not adversely affected by interferences from common hemoglobin variants in samples.	A disadvantage of the enzymatic method is its relatively high cost.

Conclusion

Globally, the incidence and prevalence of type 1 and type 2 diabetes mellitus (DM) has increased significantly over the past two decades and is expected to continue to increase in the future. Diabetes is associated with several chronic complications that lead to increased morbidity and mortality. HbA1c is an accurate and easy-to-manage test with onsite results availability. It can be an effective tool for diagnosing and prognosis of diabetes, especially in low- and middle-income countries and in hard-to reach populations. Hyperglycemia is a key biochemical feature of diabetes that should be rigorously controlled and maintained in a range as close to normal as possible to mitigate the risk of diabetic complications. Both the level of and exposure to hyperglycemia, as well as glycemic variability, contribute to the pathogenesis of diabetic complications, with different patterns of disease pathogenesis in patients with type 1 or type 2 diabetes. Despite its analytical and biological limitations, HbA1c

remains the key biomarker of long-term glycemic control. However, it has become apparent in recent years that other glycated proteins, 1,5-AG, and integrated measures from direct glucose testing by SMBG/CGMS may provide valuable data complementary to HbA1c, particularly in circumstances when the HbA1c results may be unreliable or insufficient to assess the risk of adverse outcomes Long-term associations of these alternative biomarkers of glycemia with the risk of diabetic complications need to be investigated to provide clinically relevant cut-off values and validate their utility in diverse populations of patients with diabetes. Better understanding of the limitations of HbA1c may improve prediction and diagnostic accuracy, much needed in order to identify those individuals who require fast track into lifestyle modification and/or other therapeutic programs currently, used as a complementary test to FPG or OGTT remains likely to be the approach that is most beneficial.

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Ethical Clearance- Not Required

Conflict of Interest- Nil

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