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A Review on Role of HbA1c in Secondary Effects of Diabetes Mellitus

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ABSTRACT

The prevalence of diabetes in population and type 2 diabetes is particular, has reached epidemic proportions worldwide. The HbA1c test is currently one of the best ways to diagnose diabetes. Measurement of glycated hemoglobin is recommended for both (a) checking blood sugar control in people who might be pre-diabetic and (b) monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. Measurement of HbA1c is an estimation of long term average glycemia, which assists diabetics as well as their physicians by providing treatment goals to reduce the risks associated with the development and progression of chronic complications of diabetes. Studies have shown that A1c is an index of average blood glucose over the preceding few weeks to months. HbA1c truly does not reflect glycemic control as claimed. There are many factors that cause variation in A1c results. Factors affecting measurement of HbA1c like erythrocyte turnover rate, alcoholism, use of certain drugs in treatment of malignancies, human immunodeficiency virus or hepatitis C virus infection are known provide false results. Hb variants are formed by single base pair mutations in the globin genes of hemoglobin, resulting in an amino acid substitution. There are over 700 silent variants of which most of them interfere with HbA1c. The measurement of HbA1c is usually by HPLC method, but interference of these Hb variants is known to provide false results, hence it is recommended to modify the method to get accurate results.

Keywords: HbA1c, Diabetes, Glycemic control.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by rise in blood glucose level called "hyperglycaemia" (Aaron & vinik, 2001). The worldwide prevalence of diabetes in 2000 was approximately 2.8% and is estimated to grow to 4.4% by 2030. This translates to a projected rise of diabetes from 171 million in 2000 to well over 350 million in 2030.Diabetes is of two types, type 1 accounting for 5%prevalence and type 2 for 95% prevalence among diabetics. This calls for improved treatment of hyper glycemic and other risk factors associated with metabolic syndrome. Since it is possible to dramatically lower the risk of both micro and macro vascular complications (Gaede P *et al*., 2003). Persistent elevations in blood sugar increase the risk for the long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, erectile dysfunction, neuropathy (loss of sensation, especially in the feet), gangrene and gastro paresis (slowed emptying of the stomach). Poor blood glucose control also increases the risk of short-term complications of surgery such as poor wound healing.

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Glycated hemoglobin (*glycosylated hemoglobin, hemoglobin A1c, HbA1c, A1c*, or *Hb1c*; sometimes also *HbA1c*) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose (Larsen ML, Hørder M, 1990). The measurement of glycosylated hemoglobin (GHb) is one of the well established means of monitoring glycemic control in patients with diabetes mellitus (Gaede P *et al*., 2003). The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was first proposed in 1976 (Koenig RJ *et al*., 1976). The clinical significance of HbA1c test was cemented by Diabetes Control and Complications Trial (DCCT) in type 1 diabetes4 and the United Kingdom Prospective Diabetes Study (UKPDS) in type 2 diabetes (UKPDS, 1998). The studies showed that HbA1c is an important marker in assessing a patient's risk of micro vascular complications and hypoglycemia. Hence, measurement of both HbA1c and blood glucose levels are now used in the routine management of patients with type 1 and type 2 diabetes (American Diabetes Association, 2006). Measurement of Glycated hemoglobin is recommended for both (a) checking blood sugar control in people who might be pre-diabetic and (b)

monitoring blood sugar control in patients with more elevated levels. According to the American Diabetes Association guidelines the glycosylated hemoglobin test can be performed at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly.

FORMATION OF GLYATION PRODUCTS: HbA1c

Excessive production of the early glycation products is an acute reversible change induced by hyperglycemia. These glycation products are formed both inside and outside the cells, as glucose rapidly attaches to the amino groups of the proteins through the non enzymatic process of nucleophilic addition, to form Schiff base adducts. Within hours, these adducts reach equilibrium levels that are proportional to the blood glucose concentration and subsequently undergoes rearrangement to form an more stable early glycation products, which reach equilibrium over a period of several weeks. One of the proteins Glycated in this way is glycated haemoglobin8. Once a hemoglobin molecule gets Glycated, a buildup of glycated hemoglobin within the red cell therefore reflects in patients with diabetes whose therapy has changed or who are not meeting glycemic goals (American Diabetes Association, 2006). Once a hemoglobin molecule gets glycated, a buildup of glycated hemoglobin within the red cell therefore reflects the average level of glucose to which the cell has been exposed during its life cycle. Measuring Glycated hemoglobin can assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The HbA1c level is proportional to average blood glucose concentration over the previous four weeks to three months. Some researchers state that the major proportion of its value is related to a rather shorter period of two to four weeks (Michigan Diabetes Research & Training Center., 2007). Excessive formation of early glycation products may adversely affect several functions relevant to diabetic complications. In blood vessels, uptake of LDL may be enhanced; resulting in atherogenesis (Witzum JL *et al*., 1982), and also the free radical mediated damage is increased (Gillery P *et al*., 1988). These reversible biochemical abnormalities probably play a role in the pathogenesis of the early functional changes in the diabetic microvasculature. Hence Measurement of HbA1c, as an estimate of long-term average glycemia, assists diabetics as well as their physicians by providing treatment goals to reduce the risks associated with the development and progression of chronic complications of diabetes (Sacks DB *et al*., 2002).

CORRELATION BETWEEN HbA1c AND PLASMA GLUCOSE

The relationship between A1c and Plasma glucose (PG) is complex. Higher levels of HbA1c are found in people with persistently elevated blood sugar, as in diabetes mellitus. A diabetic person with good glucose control has an HbA1c level that is close to or within the reference range. The International Diabetes Federation and American College of Endocrinology (IDFACE) recommend HbA1c values below 6.5%, while American Diabetes Association (ADA) recommends that the HbA1c be below 7.0% for most patients (Diabetes Care 2009). On average, HbA1c of 6% corresponds to mean plasma glucose of 135 mg/dl. For every increase in A1c of 1%, mean plasma glucose increases by 35 mg/dl. A normal non-diabetic HbA1c is 3.5-5.5%. In diabetes about 6.5% is good. Many studies have shown that A1cis an index of mean plasma glucose over the preceding weeks to months. A1c truly does not reflect glycemic control over last three months as it is claimed. Rather, it is weighted to the more recent weeks. The mean glycemia during the month preceding the A1c measurement contributes 50% of the result, during the 30-60 days prior to the A1c measurements contributes another 25% and during the 60- 120 days prior to the measurement contributes the final 25% (Beach KW, 1979). HbA1c values corresponding to glucose level is given in Table.1. The approximate mapping between HbA1c values and eAG (estimated Average Glucose) measurements is given by the following equation (Nathan DM *et al*., 2008).

$$eAG(mg/dl) = 28.7 \times A1c - 46.7$$

$$eAG(mmol/l) = 1.59 \times A1c - 2.59$$

A borderline (5.6–6.4%) or high (≥6.5%) level of HbA1c was found to strongly predict future drug treatment for Diabetes mellitus. (Tamae Shimazaki *et al*., 2007).

Table.1 HbA1c values corresponding to glucose level

HbA_{1c}	eAG (estimated Average Glucose)	
(%)	(mmol/L)	(mg/dL)
5	5.4 (4.2–6.7)	97 (76–120)
6	7.0 (5.5–8.5)	126 (100–152)
7	8.6 (6.8–10.3)	154 (123–185)
8	10.2 (8.1–12.1)	183 (147–217)
9	11.8 (9.4–13.9)	212 (170–249)
10	13.4 (10.7–15.7)	240 (193–282)
11	14.9 (12.0–17.5)	269 (217–314)
12	16.5 (13.3–19.3)	298 (240–347)

Data in parentheses are 95% Confidence intervals

QUANTIFICATION OF HbA1c

In measurement of HbA1c the prevalence of the most common hemoglobin variants (HbS, HbC, and HbD) depends on the genetic background of the population being analyzed. Single base pair mutations in the globulin genes of hemoglobin, resulting in an amino acid substitution. More than 700 Hb variants are known and about half of these variants are clinically silent variant (Alphabetical hemoglobin variant list [editorial] 2006). The presence of these Hb variants may falsely interfere with measurement of HbA1c by HPLC (Schnedl WJ *et al*., 2001). Hence the identification of Hb variants is important to avoid inaccurate HbA1c results. The degree of interference of Hb variants may vary with each method and even with each method modification. The ‘‘true’’ HbA1c results may be obtained after appropriate correction based on the peak area for each glycated and

non glycated component separated in the chromatograms. The HPLC method used by Camargo HPLC (Merck-Hitachi L-9100 Glycated Haemoglobin Analyzer; Tokyo, Japan) using a CCMpack Hb-S column in high speed mode. This cation exchange column allows separation of Hb variants and the calculation for the glycated component is only related to HbA1c, not to HbS1c, HbC1c or to HbD1c resulting in very low GHbvalue. Hence it is recommended that laboratories measure. GHb by methods that is not affected by Hb variants, rather than estimate it (Camargo JL *et al*., 2004).

The development of automated HPLC method modification with high resolution mode aids the identification of interference caused by clinically silent hemoglobin variants in glycated haemoglobin (HbA1c) determination. Most HPLC systems are not able to resolve additional peaks in their chromatograms and this leads to the overestimation and underestimation of HbA1c results (Lahousen T *et al*., 2002). Affinity chromatographic methods measure glycol hemoglobin regardless of the glycation site and result in more accurate measure of glycaemic control in samples with haemoglobin variants produced by hemoglobin mutations. (Schnedl WJ *et al*., 2000). New methods are being developed, such as electrospray mass spectrometry (Nakanishi T *et al*., 2000) and a method based on quenching of the fluorescence of an eosin-boronic acid solution (Blincko S *et al*., 2000). The immune agglutination method used was the DCA 2000 (Bayer, Vienna, Austria), which uses a specific antibody against the first six amino acid residues of the glycated Nterminal of hemoglobin. It was determined that only a few Hb variants are known to interfere with HbA1c results in immunoassays (Bry L *et al*., 2001)

LIMITATIONS OF CORRELATION BETWEEN HbA1c AND PLASMA GLUCOSE LEVEL

HbA1c is an important and useful parameter for the treatment of diabetes, but there are several problems relating to its use for diagnosis. It varies not only according to the level of glycaemia but also to the turnover rate of hemoglobin. (Takeshi Kuzuya *et al*., 2002). HbA1c level can be lower than the expected in people with shortened red blood cell life span, sickle-cell disease or any other condition that could result in premature red blood cell death. Lower A1c levels are found in diabetic and non diabetic pregnant women, probably due both to lower fasting blood glucose and a shortened erythrocyte lifespan (Nielsen LR *et al*., 2002). Vitamins C and E have been reported to lower A1c measurements, possibly by inhibiting glycation (Davie SJ *et al*., 1992). Concomitant use of many drugs to treat patients with malignancies, human immunodeficiency virus or hepatitis C virus infection, may have a GHb lowering effect and produce an negative result in the patients with diabetes. Alcoholism, lipademia, and chronic ingestion of salicylates, is also known to reduce the level of HbA1. HbA1c level can be higher than expected levels can be seen in people with a longer red blood cell life span, such as with Vitamin B12 or folate deficiency. There is also some evidence of wide fluctuations in HbA1c between individuals that are unrelated to glycemic status, suggesting that there are "low glycators" and "high glycators (Kilpatrick ES *et al*., 1998). In a study where the HbA1c and fructosamine levels are simultaneously measured, showed that the HbA1c does not accurately reflect glucose control and it was suggested for the measurement of fructosamine to be routinely used in diabetes practice (David R Macdonald *et al*., 2008).

CONCLUSION

Hemoglobin A1c (HbA1c) has been widely used as a measure of glycemic control in patients with diabetes mellitus. It can be used to estimate long-term average glycaemia and design dosage regimen for diabetes mellitus. However measurement of HbA1c level as a sole tool for assessing the diabetic status cannot be used clinically since level of glycalated Hb depends on various reasons as mentioned above and may give rise to false results. Hematological status should always be considered to ensure the correct interpretation of GHb results. HbA1c levels are measured by HPLC methods routinely and the presences of Hb variants are known to interfere with the HbA1c levels and cause false result. Hence the modification of the HPLC method enables interference with HbA1c determination as a result of silent hemoglobin variants can be recognized more easily. It is recommended that laboratories measure GHb by a method that is not affected by Hb variants.

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