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Using Glycated Hemoglobin HbA1c for diagnosis of Diabetes mellitus: An Indian perspective.

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ABSTRACT

Glycated Hemoglobin (HbA1c) gives an estimate of long-term average glycemic status. It is used routinely to assess glycemic control in diabetics to attain treatment goals and prevent long term complications. Its recommendation for diagnosis of diabetes mellitus has evoked mixed response worldwide. We reviewed a number of published articles to analyze the pros and cons of using HbA1c for diagnosis of Diabetes mellitus in India. We observed that though HbA1c has some indisputable advantages over fasting plasma glucose estimation for diagnosing diabetes mellitus, a number of biochemical, clinical and economical factors limit its use as single diagnostic agent. Diagnostic methods and laboratories are insufficiently standardized for HbA1c in India. The clinician must consider the overall patient profile in addition to a number of local variations and disorders especially hemoglobinopathies /anemias before accepting an abnormal HbA1c value. Supportive or repeat tests may be required leading to increase in cost and delay in diagnosis. In the present Indian scenario, especially the fragmented unorganized health care sector in suburban areas, HbA1c cannot be accepted as a sole and independent test to diagnose diabetes mellitus.

1. Introduction

Glycated hemoglobin (GHb) is formed by a posttranslational, non-enzymatic, substrate-concentration dependent irreversible process of combination of aldehyde group of glucose and other hexoses with the amino-terminal valine of the β -chain of hemoglobin [1]. Since the time it was first described, its importance and utility for prognosis, monitoring and diagnosis of diabetes mellitus has been a matter of research and debate. This article tries to analyze the pros and cons of using HbA1c as a diagnostic marker for diagnosis of diabetes mellitus in the Indian health care system.

In 1958 Allen et. Al [2] published a paper describing the heterogeneity of haemoglobin A. The fractions that eluted at more acidic pH on the anion exchanger carboxy methylcellulose and migrated more rapidly on electrophoresis were called minor haemoglobins or fast haemoglobins. They could be sub fractionated into the species A(1a), A(1b), A(1c), A(1d). The significance of these sub-fractions was then unclear and often interpreted as artefacts or insignificant. This radically changed in 1968 when Samuel Rahbar reported on a survey of 1,200 hospital patients that 2 diabetic patients in this group had a fast-moving haemoglobin on starch gel electrophoresis [3]. A further 47 diabetic subjects including 11 children with severe diabetes mellitus also had this haemoglobin fraction. Later this fast haemoglobin was identified as Allen's HbA1c and the charge difference localised to the β chain [4]. Homquist et. al. [5] had published on the β chain N terminally blocking group of HbA1c but the definitive structure was elucidated by Bunn et. al. [6] The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and co-workers [7].

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The Prognostic role of HbA1c is well established and accepted. In the normal 120-day lifespan of the red blood cell, glucose molecules react with hemoglobin, forming glycated hemoglobin. Glucose forms an aldimine linkage with NH2- of valine in the β-chain, undergoing an Amadori rearrangement to form the more stable ketoamine linkage. Glycated hemoglobin has been used primarily to as a marker to identify the average plasma glucose concentration over prolonged periods of time. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, and retinopathy.

Traditionally, HbA1c has been thought to represent average glycemia over the past 12 to 16 weeks [8]. In fact, glycation of hemoglobin occurs over the entire 120-day life span of the red blood cell [9] but within these 120 days recent glycemia has the largest influence on the HbA1c value [10]. Kinetic studies have revealed that glycemia in the recent past influences the GHb values more than the remote past [11]. Thus, mean blood glucose of past 1 month, 2 months and 3 months contributes 50%, 40% and 10% respectively to the final result. By mathematical modelling the t1/2 of HbA1c is estimated to be 35.2 days [12]. This means that half of glycation seen during estimation has occurred in the previous 35.2 days. The advantage that HbA1c can give as an assessment of average plasma glucose can also be perceived as a drawback because it does not give an indication of the stability of glycemic control. Thus, in theory, one patient with wildly fluctuating glucose concentrations could have the same HbA1c value as one whose glucose varies little throughout the day. The International Diabetes Federation and American College of Endocrinology recommend HbA1c values below 6.5%, while American Diabetes Association recommends that the HbA1c be below 7.0% for most patients.[12] .Practitioners must consider an individual patient's health, his/her risk of hypoglycemia, and his/her specific health risks when setting a target A1C level. Patients at high risk of microvascular complications may gain further benefits from reducing A1C below 7%. Because patients are responsible for averting or responding to their own hypoglycemic episodes, the patient's input and the doctor's assessment of the patient's self-care skills are also important.
The approximate mapping between HbA1c values and eAG (estimated average glucose) measurements is given by the following equation:[13]

$$eAG(mg/dl) = 28.7 \times A1C - 46.7$$
$$eAG(mmol/l) = 1.59 \times A1C - 2.59$$

The American Diabetes Association guidelines are similar to others in advising that the glycosylated hemoglobin test be performed at least two times a year in patients with diabetes that are meeting treatment goals (and that have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or that are not meeting glycemic goals[14]

DIAGNOSIS OF DIABETES MELLITUS:

Historically, the measurement of glucose has been the means of diagnosing diabetes. Type 1 diabetes has a sufficiently characteristic clinical onset, with relatively acute, extreme elevations in glucose concentrations accompanied by symptoms, such that specific blood glucose cut points are not required for diagnosis in most clinical settings. On the other hand, type 2 diabetes has a more gradual onset, with slowly rising glucose levels over time, and its diagnosis has required specified glucose values to distinguish pathologic glucose concentrations from the distribution of glucose concentrations in the non-diabetic population.

When selecting the threshold glucose values, the National Diabetes Data Group (NDDG) acknowledged that "there is no clear division between diabetics and nondiabetics in the FPG concentration or their response to an oral glucose load," and consequently, "an arbitrary decision has been made as to what level justifies the diagnosis of diabetes" which has been used for two decades [15]. The diagnosis of diabetes was made when 1) classic symptoms were present; 2) the venous FPG was ≥140 mg/dl (≥7.8 mmol/ l); or 3) after a 75-g glucose load, the venous 2HPG and levels from an earlier sample before 2 h were ≥200 mg/dl (≥11.1 mmol/l). In 1997, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [16] re examined the basis for diagnosing Diabetes. In comparing the relationship between FPG and 2HPG values and retinopathy, it was apparent that the previous FPG cut point of 140 mg/dl (7.8mmol/l) was substantially above the glucose level at which the prevalence of retinopathy began to increase . As a result, the committee recommended that the FPG cut point be lowered to ≥126 mg/dl (7.0 mmol/l) so that this cut point would represent a degree of hyperglycemia that was "similar" to the 2HPG value and diagnosis with either measure would result in a similar prevalence of diabetes in the population. The 1997 report also recommended that the FPG level, rather than the 2HPG, be the preferred test to diagnose diabetes because it was more convenient for patients and less costly and time consuming and the repeat-test reproducibility was superior [16]

HBA1C FOR DIAGNOSIS OF DM:

Chronic hyperglycemia sufficient to cause diabetes-specific complications is the hallmark of diabetes. Common sense would dictate that laboratory measures that capture long-term glycemic exposure should provide a better marker for the presence and severity of the disease than single measures of glucose concentration. Studies consistently demonstrated a strong correlation between retinopathy and A1C (17 –19) but a less consistent relationship with fasting glucose levels [20]. The correlation between A1C levels and complications has also been shown in the setting of controlled clinical trials in type 1 [21] and type 2 [22] diabetes, and these findings been used to establish the widely accepted A1C treatment goals for diabetes care [23].

Large volume of data from diverse populations has now established an A1C level associated with an increase in the prevalence of moderate retinopathy and provides strong justification for assigning an A1C cut point of ≥6.5% for the diagnosis of diabetes This cut point should not be construed as an absolute dividing line between normal glycemia and diabetes; however, the A1C level of 6.5% is sufficiently sensitive and specific to identify individuals who are at risk for developing retinopathy and who should be diagnosed as diabetic. The A1C level is said to be least as predictive as the current FPG and 2HPG values. In selecting a diagnostic A1C level ≥6.5%, the International Expert Committee balanced the stigma and costs of mistakenly identifying individuals as diabetic against the minimal clinical consequences of delaying the diagnosis in someone with an A1C level ≥6.5%.

An International Expert Committee, after an extensive review of both established and emerging epidemiological evidence, recommended the use of the A1C test to diagnose diabetes,with a threshold of ≥6.5%, and ADA affirms this decision. The diagnostic A1C cut point of 6.5% is associated with an inflection point for

retinopathy prevalence, as are the diagnostic thresholds for FPG and 2-h PG. The diagnostic test should be performed using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. Point-of-care A1C assays are not sufficiently accurate at this time to use for diagnostic purposes.

ADA 2010 Criteria for the diagnosis of diabetes: [24]

1. A1C ≥6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.* OR
2. FPG ≥126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.* OR
3. 2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*OR
4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/l).

   *In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.

Advantages of Hb A1c as recommended means of diagnosing Diabetes

After the ADA recommended HbA1C for diagnosis of Diabetes in 2010, it is gradually being accepted for the same worldwide. With advances in instrumentation and standardization, the accuracy and precision of A1C assays at least match those of glucose assays. The measurement of glucose itself is less accurate and precise than most clinicians realize [25]. There are also potential pre-analytic errors owing to sample handling and the well-recognized lability of glucose in the collection tube at room temperature [26, 27]. Even when whole blood samples are collected in sodium fluoride to inhibit in vitro glycolysis, storage at room temperature for as little as 1 to 4 h before analysis may result in decreases in glucose levels by 3–10 mg/dl in non diabetic individuals [26,27,28,29]. By contrast, A1C values are relatively stable after collection [30], and the recent introduction of a new reference method to calibrate all A1C assay instruments should further improve A1C assay standardization in most of the world [31,32,33]. The variability of A1C values is also considerably less than that of FPG levels, with day-to-day within-person variance of <2% for A1C but 12–15% for FPG [34,35,36]. The convenience for the patient and ease of sample collection for A1C testing (which can be obtained at any time, requires no patient preparation, and is relatively stable at room temperature) compared with that of FPG testing (which requires a timed sample after at least an 8-h fast and which is unstable at room temperature) support using the A1C assay to diagnose diabetes. Compared with the measurement of glucose, the A1C assay is at least as good at defining the level of hyperglycemia at which retinopathy prevalence increases; has appreciably superior technical attributes, including less preanalytic instability and less biologic variability; and is more clinically convenient. A1C is a more stable biological index than FPG, as would be expected with a measure of chronic glycemia levels compared with glucose concentrations that are known to fluctuate within and between days.

In short it provides a better index of overall glycemic exposure and risk for long-term complications, with less biologic variability, less preanalytic instability with no need for fasting or timed samples and and is relatively unaffected by acute (e.g., stress or illness related) perturbations in glucose levels

Limitations of HbA1c as recommended means of diagnosing Diabetes in India:

The most important limitation in India is the cost of providing the assay for its routine use. Second, any condition that changes red cell turnover, such as haemolytic anemia, chronic malaria, major blood loss, glucose-6-phosphate dehydrogenase deficiency, sickle cell anemia or blood transfusions, will lead to spurious A1C results. These conditions including the thlassaemias are highly prevalent in certain parts of India. Besides, Hereditary persistence of fetal Hb, renal insufficiency,malignancy, iron deficiency anemia, vitamin B 12 and folate deficiency, splenectomy also show increased values [37,38,39]. Some studies have shown that alcoholism, lead poisoning, opiate addiction , excessive use of salicylate and pregnancy can lead to falsely elevated HbA1c. Age and regional differences do exist in values of HbA1 which have not been studied widely in India. We do not have sufficient data on whether Indians are high glycaters or low glycaters [40]. HbA1c assay results cannot be trusted In certain rare clinical settings, such as rapidly evolving type 1 diabetes, where the A1C level will not have had time to "catch up" with the acute elevations in glucose levels [29].

HbA1c test are performed using different methods like High performance liquid chromatography, affinity chromatography, cation exchange chromatography, isoelectric focussing, radioimmunoassay, spectrophotometric assay, electrophoresis and electrospray mass spectrometry. Tests to diagnose diabetes should be performed using clinical laboratory equipment using a method that is NGSP certified and standardized to the DCCT assay [41]. Point-of-care instruments have not yet been shown to be sufficiently accurate or precise for diagnosing diabetes. Looking at the enormous variation in the health care system in India, labs and methods used for estimation appear to be far from standardized. With dearth of accredited labs and limited resources, the routine use of HbA1c is questionable. It would not be practical to have HPLC as the only method for HbA1c assessment to be used for diagnostic puposes. Also according to Rancho Bernardo study, the HbA1C cut point of 6.5% had a sensitivity/specificity of 44/79%. In their cohort of older adults, the suggested HbA1C cut point of 6.5% had relatively low sensitivity and specificity for type 2 diabetes diagnosis in all age-groups and in both sexes. They concluded that the limited sensitivity of the A1C test may result in delayed diagnosis of type 2 diabetes, while the strict use of ADA criteria may fail to identify a high proportion of individuals with diabetes by HbA1C 6.5% or retinopathy [42]. Also in another study by Cavagnolli et al HbA1c ≥ 6.5% (48 mmol/mol) showed limited sensitivity to diabetes diagnosis, although with high specificity. The results suggest that this cut-off point would not be enough to diagnose diabetes. They concluded that Its use as the sole diabetes diagnostic test should be interpreted with caution to assure the correct classification of diabetic individuals [43].The decision about which test to use to assess a specific patient for diabetes should be at the discretion of the health care professional, taking into account the availability and practicality of testing an individual patient or groups of patients.

As with most diagnostic tests, a test result diagnostic of diabetes should be repeated to rule out laboratory error, unless the diagnosis is clear on clinical grounds, such as a patient with classic symptoms of hyperglycemia or hyperglycemic crisis. It is preferable that the same test be repeated for confirmation, since there will be a greater likelihood of concurrence in this case. In case of non confirmation by repeat testing the healthcare professional should opt to follow the patient closely and repeat the testing in 3– 6 months. Clinicians should continue to use the

previously recommended approaches to diagnose diabetes based on glucose measurements. The decision to change to A1C assays as the means of diagnosing diabetes should take into account the performance of local A1C assays and the local prevalence of conditions that may interfere with the assay. Clinicians must be aware of these conditions, particularly in populations in which they are more prevalent.

If A1C testing is not possible owing to patient factors that preclude its interpretation (e.g., hemoglobinopathy or abnormal erythrocyte turnover) or to unavailability of the assay, previously recommended diagnostic measures (e.g., FPG and 2HPG) and criteria should be used. Mixing different methods to diagnose diabetes should be avoided. The diagnosis of diabetes during pregnancy, when changes in red cell turnover make the A1C assay problematic, continues to require glucose measurements.

The risk for diabetes based on levels of glycemia is a continuum. Therefore; there is no lower glycemic threshold at which risk clearly begins. Those with A1C levels below the threshold for diabetes but > 6.0% should receive demonstrably effective preventive interventions. Those with A1C below this range may still be at risk and, depending on the presence of other diabetes risk factors, may also benefit from prevention efforts. The A1C level at which population-based prevention services begin should be based on the nature of the intervention, the resources available, and the size of the affected population.

Conclusion:

The major fraction of the healthcare system in India is a fragmented and unorganized private sector, ranging from corporate hospitals to small clinics and private practitioners [44]. Very few laboratories performing the tests have been standardized [45]. After the ADA 2010 recommendation, there has been a gradual increase in acceptance of HbA1c as a diagnostic test for diabetes mellitus. But the clinicians prescribing and interpreting the tests results are likely to miss the numerous limitations, precautions and variations of using HbA1c for diagnosis of diabetes mellitus. Simply speaking every single HbA1c report must be correlated with the method and lab used. Any disorder of red blood cells or haemoglobin must be excluded and all local interfering factors discussed above must be taken into account. Besides a repeat HbA1c testing or plasma glucose estimation is usually recommended before abnormal values can be accepted. All this involves an increased cost and delay in diagnosis. This might not be a limitation in large organized and standardized city hospitals. But in the present Indian scenario Glycated haemoglobin, HbA1c cannot be accepted as a sole and independent test to diagnose diabetes mellitus.

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