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REVIEW ARTICLE

Importance and Status of HBA1C in T2DM and its Indian Perspective

Devajit Sarmah*, Booloo Sharma

R. D. Gardi Medical College, Agar Road, Surasa, Ujjain, MP (India)

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ABSTRACT

Type-2 diabetes is a common non-communicable disease, especially in developing countries like India, posing a huge economic burden on the family and nation as a whole. Traditionally fasting plasma glucose and OGTT is advocated for diagnosing type-2 diabetes, and HbA1c is often considered for monitoring. Very recently however HbA1c is proposed for diagnosis of HbA1c, acceptance of which is greatly debated. This article describes the evolution of HbA1c from the time of its discovery to the present status of a diagnostic test for type-2 diabetes. We observed that HbA1c has some major advantages over fasting plasma glucose estimation for diagnosing diabetes mellitus, although various biochemical and clinical factors act as a limitation. These limitations can be nullified very often, but the lack of laboratory standardization of method and the cost of testing limits its utility in India. But we conclude that HbA1c can be considered once effective cut off levels are established and laboratories are standardized, as the huge economic burden which diabetes pose in India over comes the cost of HbA1c testing in India.

Keywords: HbA1c, India

1. INTRODUCTION

Diabetes mellitus has made its presence since time immoral, and through the ages people knows it by various and diverse terminologies. Diabetes mellitus was known to Indian physician as early as 2500 BC when it was known as “madhumeha”. Presently, the term diabetes mellitus is coined for a conglomeration of metabolic disorders of various etiologies which is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. Of all the cases of diabetes mellitus, almost 90% are of type 2 diabetes mellitus (T2DM) and rest being type 1 (T1DM). Untreated diabetes ultimately leads to microvascular and macrovascular complications, more so in T2DM [2].

Worldwide the prevalence of T2DM has been rising, and more than 180 million people are affected globally. The noteworthy fact is that epidemics of diabetes mellitus have taken place in developing countries [3, 4]. The major global diabetic load occurs in India and China, where more than 75% of diabetic subjects will live by the year 2025 [5], and by then every fifth diabetic subjects in the world would be an Indian [6]. The dramatic economic changes have had a great impact on urbanization and lifestyle of the Indians, which together with genetic predisposition contributed to the rise in diabetes in India [7]. Also the presentation of T2DM occurs a decade earlier in Indians when compared to European population [8]. Even complication like CAD occurs at an early age in Indians with T2DM [9].

Early diagnosis and efficient management of diabetes mellitus has been advocated by various association and organization. Among the battery of test recommended, fasting plasma glucose (FPG), 2 hr oral glucose tolerance test (OGTT), random plasma glucose (RPG) and HbA1c are advocated. HbA1c is noteworthy because it can be used for both diagnosis of T2DM (bearing some exception), as well as in prognosis. The present article is an attempt to discuss the importance and the status of HbA1c in diagnosis and its implication in the Indian health care system.

2. HISTORY OF HbA1c

HbA1c is formed when aldehyde group of glucose and some other hexoses combine irreversibly, posttranslationally and non-enzymatically with the amino-terminal valine of the β -chain of hemoglobin, and this process is substrate-concentration dependent [10]. The heterogeneity of haemoglobin A was first described in 1958 by Allen et. Al [11]. Then between 1958 and 1961, Allen, Schroeder and colleagues chromatographically separated several fast moving minor Hb components in red cell haemolysates from healthy adults [11-13]. They were called minor haemoglobins or fast haemoglobins because of their fast migration in elctrophoretic field. They were described as HbA1a, HbA1b, HbA1c, in the order in which

*Corresponding author: Dr. Devajit Sarmah| Mobile: +91-8120926967| Email: dr.devajit@gmail.com

they were eluted. Though in 1962 Huisman and Dozy noted an elevation of these "fast" moving haemoglobins in diabetic individuals, they attributed it to tolbutamide treatment in their patients [14].

So, fast hemoglobin were interpreted as artifacts until when Samuel Rahbar in 1968 and again in 1969 found an increase in fast-moving haemoglobin in diabetic patients [15], and finally Trivelli in 1971 suggested the relationship between mean blood glucose, long-term diabetic complications and fast haemoglobins [16].

Koenig et al, in 1975, using genetically modified diabetic mice reported that the HbA1c fraction was present in diabetes after the onset of diabetes, which appeared to occur post-translationally [17].

Subsequently various other workers also identified this fast haemoglobin in diabetic patient which was named as Allen's HbA1c and it was found that the charge difference is localised to the β chain [18]. The definitive structure of HbA1c was finally elucidated by Bunn et. al. [19].

Finally, it was Anthony Cerami, Ronald Koenig and co-workers, who in 1976 proposed the role of HbA1c for monitoring the degree of control of glucose metabolism in diabetic patients [20]. This early report stated that, "The periodic monitoring of HbA1c levels provides a useful way of documenting the degree of control of glucose metabolism in diabetic patients and provides a means whereby the relation of carbohydrate control to the development of sequellae can be assessed"[20].

Glucose forms an aldimine linkage with NH2- of valine in the β- chain, undergoing an Amadori rearrangement to form the more stable ketoamine linkage. This process known as glycation of hemoglobin occurs in a span of 120 days, which is the life span of a normal RBC [21]. The process of glycation depends on the average glucose load during the period of RBC life span, and so the role of HbA1c was rightly accepted for monitoring of plasma glucose concentration over prolonged periods of time.

Though traditionally, HbA1c has been thought to represent average glycemia over the entire 120-day life span of the red blood cell but it is described that recent glycemia has the largest influence on the HbA1c value [22]. So it is the recent glucose concentration which mostly affects the measured HbA1c as revealed by kinetic studies [23]. It has been verified that, the mean blood glucose of preceding 1 month, 2 months and 3 months contributes 50%, 40% and 10% respectively to the final result. Thus, mathematically $t_{1/2}$ of HbA1c is estimated to be 35.2 days [24], and this means that half of the glycation has occurred in the previous 35.2 days. HbA1c thus, does not predict the stability of glucose concentration as a recent change in glucose concentration affects the HbA1c value more and can sometimes be misleading especially in subjects having recent fluctuation in glucose load. In spite of this fact the prognostic role of HbA1c is well established and accepted. The development of chronic vascular complication of diabetes like retionopathy, nephropathy and cardiovascular disease is intimately linked to the level of glycemic control attained by the individual with diabetes and so monitoring of glycemia is of utmost importance, and HbA1c is the only means for monitoring of average glycemia. The International Diabetes Federation and American College of Endocrinology including ADA recommend HbA1c values below 6.5 for most patients [25-28].

3. DIGNOSIS OF DIABETES

Ever since diabetes was recognized, plasma glucose became the criteria for the diagnosis of diabetes. It was either the fasting plasma glucose (FPG), or the 2-h value in the 75-g oral glucose tolerance test (OGTT). Gradually HbA1c came into the picture and its potentiality was recognized by W.H.O. in 1985 in diabetic management and sugar monitoring. But then, it was in 2009, when an International Expert Committee that included representatives of the American Diabetes Association (ADA), the International Diabetes Federation (IDF), and the European Association for the Study of Diabetes (EASD) recommended the use of the A1C test to diagnose diabetes, with a threshold of ≥6.5% (48 mmol/mol) [29], and ADA adopted this criterion in 2010. This value is apparently selected because it is found that above this value the incidence of retinopathy is increased. This is considered important because retinopathy, a common complication of diabetes is often present even before the actual diagnosis of diabetes is made.

4. ADA 2010 Criteria for the diagnosis of diabetes [29]:

1. HbA1c ≥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.

5. HbA1c IN THE DIAGNOSIS OF DIABETES

The potential utility of HbA1c in diabetes care is first mentioned in the 1985 W.H.O. report [30]. And by 2010 all the major expert committee and association across the globe including the ADA has recommended HbA1c for the

diagnosis of type 2 diabetes. But, ADA also clarifies, that HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardized to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement. This clarification is important because the usefulness of HbA1c is criticized primarily for its lack of sensitivity and for the confounding aspects of assay and reference -range standardization and of inadequate quality control [31, 32].

From the proposed ADA guideline for diagnosis of diabetes it is clear that FPG, OGTT and HbA1c are used in the diagnosis of diabetes. All the three parameters possess their own characteristic pros and cons regarding their utility for diagnosis of diabetes. This article tries to discuss the pros and cons of HbA1c in the diagnosis of diabetes, and in doing so it draws comparisons of HbA1c with FPG and OGTT.

Fasting plasma glucose is defined as testing blood sugar after no calorie intake for 8 hr, and hence compliances are poor. In a study where 5,752 of 8,286 eligible participants were screened for diabetes, only in 3% FPG is performed (152) and in 95% of participants random plasma glucose was analyzed [33]. Patient preparation is also required for measurement of oral glucose tolerance test (OGTT), and the cumbersome techniques of OGTT makes it not so favorite test among the clinician as well as diabetic subjects. In contrast the HbA1c testing is devoid of any such preparation, and bears the advantage of performing in any time of the day.

Fasting plasma glucose are altered by numerous factors like stress, acute illness, medication, venous stasis, posture, sample handling, food ingestion, prolonged fasting and exercise [34]. These factor, are also likely affects the 2 hr OGTT. The same factors, however does not have any affects on HbA1c measurements.

In case of fasting blood glucose intraindividual variation in healthy persons is reported to be 5.7 – 8.5%, while interindividual variation is revealed to be upto 12.5% [35, 36]. Also there is high degree of intraindividual variability in the OGTT, with a CV of 16.7%, which is considerably greater than the variability for FPG [35]. Thus the reproducibility of the OGTT is poor [37, 38]. But compared to these two parameters used for diabetic diagnosis, HbA1c shows a relatively lower biological variation with a CV <1% [39].

Stability of glucose measurement is always a major aspect to be considered in measuring FPG. Glycolysis consumes glucose even in fluoride preservative for the first two hours after blood is collected, and may continue upto 4 hrs [40]. The delay in the glucose stabilizing effect of fluoride is thought to be the result of glucose metabolism proximal to the fluoride target enolase [41]. The rate of glycolysis is also increased in samples which contain an increased concentration of RBC, WBC and platlets. This fact questions the accuracy of FPG measured and used for diagnosis of diabetes mellitus. As the same process is involved in the measurement of OGTT, like FPG its accuracy is also always questioned. In contrast HbA1c has high preanalytical stability and is stable for 1 week when stored at 4°C and for 1 year when stored at -70°C [42, 43].

Internationally recognized reference methods are not available for Glucose measurement. There is no program to standardize results among different instruments and different laboratories. Glucose is measured in laboratories almost exclusively using enzymatic methods, predominantly with glucose oxidase or hexokinase [44]. Numerous improvements in glucose measurement have produced low within-laboratory imprecision (CV 2.5%). Analysis in ~6000 laboratories using 32 different instrument revealed a statistically significant difference in glucose measurement, with bias ranging from -6 to +7 mg/dl (-6 to -7%) at a glucose concentration of 100 mg/dl [45]. HbA1c on the other hand is standardized and the National Glycohemoglobin Standardization Program (NGSP) has been instrumental in standardizing HbA1C testing among laboratories [46, 47], along with International federation of clinical chemistry (IFCC) [48, 49].Glucose can be measured in plasma, serum or whole blood, but for the diagnosis of diabetes plasma glucose is recommended by both ADA and WHO [29, 30]. In spite of the recommendation, it is a well established fact that most laboratories measures serum glucose. Various studies have also verified that plasma glucose differs from serum glucose measurement [50-53]. So serum glucose measurement many a times is used erroneously in diagnosis of diabetes and should be replaced with a FPG measurement. Whereas a single whole blood specimen is required for testing HbA1c and it is recommended universally.

FPG is subjected to diurnal variation and NHANES III data reveals this finding. Mean FPG in the afternoon was considerably lower than the morning FPG sampled as verified by NHANES III data of previously diagnosed diabetes subjects [33]. So, a FPG collected in the morning would be high compared to FPG collected in the afternoon after 8 hrs of calorie restriction, and the chances of erroneous categorization as diabetic or as IFG cannot be denied. But, HbA1c measurement can be collected at any time of the day at diabetic subjects own convenience, as its values are not subjected to diurnal variation.

Thus, the paragraphs above shows that HbA1c which predicts glycemic status in the preceding 8 to 12 week period, is a stable test with very less interference and test

does not require prior preparation. As HbA1c arises due to glycation of hemoglobin in RBC, and any factors that interferes with the process of glycation or with the concentration of hemoglobin or erythrocytes, affects the result of HbA1c test [26, 27, 54]. The below mentioned are the factors which may affect the level of measured HbA1c.

6. Some of the factors that influence HbA1c and its measurement. Adapted from Gallagher et al [54]

6.1. Erythropoiesis

Increased HbA1c: iron, vitamin B12 deficiency, decreased erythropoiesis.

Decreased HbA1c: administration of erythropoietin, iron, vitamin B12, reticulocytosis, chronic liver disease.

6.2. Altered Haemoglobin

Genetic or chemical alterations in haemoglobin: haemoglobinopathies, HbF, methaemoglobin, may increase or decrease HbA1c.

6.3. Glycation

Increased HbA1c: alcoholism, chronic renal failure, decreased intraerythrocyte pH.

Decreased HbA1c: aspirin, vitamin C and E, certain haemoglobinopathies, increased intra-erythrocyte pH.

Variable HbA1c: genetic determinants.

6.4. Erythrocyte destruction

Increased HbA1c: increased erythrocyte life span: Splenectomy**.**

Decreased HbA1c: decreased erythrocyte life span: haemoglobinopathies, splenomegaly, rheumatoid arthritis or drugs such as antiretrovirals, ribavirin and dapsone.

6. 5. Assays

Increased HbA1c: hyperbilirubinaemia, carbamylated haemoglobin, alcoholism, large doses of aspirin, chronic opiate use.

Variable HbA1c: haemoglobinopathies.

Decreased HbA1c: hypertriglyceridaemia.

*Some of the above interfering factors are "invisible" in certain of the available assays

Diabetic with additional disease like hemolytic disease or other conditions with shortened erythrocyte survival can reduce HbA1c values substantially [54]. So in case of acute blood loss HbA1c values is spuriously low because of an increased fraction of young erythrocytes. In diabetics with iron deficiency anemia there is an increase in HbA1c concentrations [55], and this levels can be reduced by therapy with iron [55, 56]. A mechanism for the higher A1C was linked to malondialdehyde, which is increased in subjects with iron deficiency anemia [55], and malondialdehyde is found to augment the process of hemoglobin glycation [57]. In the NHANES 1999–2006 population without known diabetes, mean A1C levels were equal or 0.1% higher in iron-deficient women and men, respectively, compared with their iron-sufficient counterparts [58]. As is seen the magnitude of difference in iron deficiency anemia is small, but it is advisable to correct iron deficiency anemia before HbA1c testing.

In patients with hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, or chronic ingestion of salicylates HbA1c can be falsely increased [42].

Intraindividual variation in HbA1c level is less with CV <1%. Interindividual vaiation is greater and is due to the differences in the rate of glycation of hemoglobin. They are called low and high glycators [59]. This is attributed to genetic consideration by various twin studies [60, 61]. Studies have described a significant racial difference in HbA1c concentration [62, 63]. The underlying molecular mechanism contributing to racial and ethnic differences attributed to difference in the rates of glucose uptake by RBC, rate of intra-erythrocytic glucose metabolism, rates of glucose attachment to or release from hemoglobin or RBC life span [64, 65]. A recent meta-analysis of 23 genome-wide association studies has identified 10 genomic loci associated with HbA1c. Of these 7 loci though unrelated with glycaemic pathways, influence HbA1c through their effects on iron status and red cell indices [66]. So, few studies are to be conducted to verify whether Indians are low or high glycators, and set national HbA1c range from such studies.

HbA1c values is said to be affected by age, with a 0.1% increase per decade [63, 67,68]. But this effect of age is controversial [69-71].

If the measured value of HbA1c is > 15% or if a large change in HbA1c coincides with a change in laboratory HbA1c methods, then the presence of hemoglobin variant should be considered [54]. A1C measurement is not appropriate in subjects homozygous for HbS or HbC, with HbSC or with any other variant that alters erythrocyte survival. However, A1C can be measured accurately in individuals heterozygous for HbS, HbE, HbC, or HbD and in those with increased HbF, provided an appropriate assay is used [54].

HbA1c testing is not considered in pregnancy. This is sometime attributed to a decrease in the life span of erythrocytes in pregnancy [72]. Pregnancy causes a decrease in plasma glucose partly due to fetal transfer and partly because of store as fat and glycogen to be used during later part of pregnancy. Since, erythrocytes are exposed to a lower average glucose the hemoglobin glycation is less [73].

7. DIABETES, HbA1c AND INDIA

Diabetes is the most prevalent non communicable disease in India engulfing the urban and rural India. India is depicted to contribute about 12% to an annual increase in diabetic population globally [74]. It has been depicted by several studies that awareness of chronic diseases is extremely low in rural areas [75]. It is also found that the

ratio of known to unknown diabetes is 3:1 in rural India compared to 1:1 in urban India [76]. Many recent studies have shown an increasing trend of diabetic population in rural India [77]. This can be the result of steady penetration of urbanization towards rural India, as a result of which there has been a significant change in lifestyles of rural India. Further studies have proofed that diabetes affects Indian at an early age. Also diabetes is increasing in youth and study shows that diabetes in population under 44 years has increased to 36% in 2006 compared to 25% in 2000 in southern part of India [78]. The increase in diabetes among youth infers that in another 10-20 years there could be a severe decline in the productivity of youth of our country [79, 80]. So the engulfment of the rural India and occurrence of diabetes at an early age and consequent increase in incidence among youth of India demands an early diagnosis and appropriate management of diabetes among Indians. An efficient screening and diagnostic system for diabetes is very much essential in India. Though traditionally as well as presently glucose remains the mainstay for screening and diagnosing diabetes, few Indian studies have verified the importance of HbA1c. A recent study by Kumar *et.al.* assessed the validity of HbA1c as a screening and diagnostic test for diabetes. They found that HbA1c value of 6.1% (43 mmol/ml) had optimal sensitivity and could thus be used for screening and 6.5% to have optimal specificity and could thus be used as a diagnostic test [81].

Various studies in Southern India has shown an increase prevalence of diabetes and impaired glucose tolerance (IGT) [82-85]. Also studies in Asian Indians have shown that IGT has a 55% conversion rate to diabetes in 3 years among Indians [86]. Traditionally diabetes is regarded as a "silent disease", as it exhibit no symptoms until it progresses to severe target organ damage [87]. The prevalence of retinopathy is significant in the Asian and Pacific Island nations, where approximately 30% of type 2 diabetics suffer from diabetic retinopathy [88], study from south India shows a prevalence rate of 34.1% [89]. Even a CVD rates are reported to be higher among Indian diabetics [90, 91]. Once diabetes is diagnosed, it is the inadequate glycaemic control which progress to life threatening complication [92, 93]. Undoubtedly an effective and efficient prevention and care system have the potential to lower the rate of complications, disability and premature mortality, and thereby resulting in long term saving in health expenditure [94, 95]. ADA has suggested an HbA1c value of 5.7 – 6.4 % as the high risk range. So, such high risk or IGT cases should have a regular glycaemic monitoring by HbA1c. Also Diabetic Prevention Programme (DPP) has verified that preventive interventions are effective in groups of people with HbA1c value below or above 5.9% [96]. So, as advocated by ADA and other international expert committee the HbA1c can promise to go a long way in monitoring of glycaemic status and prevention of complications among Indian diabetics. Raheja et al [92] have contributed to the literature in India, noting a positive relationship between mean HbA1c level and frequency of complications among patients with long diabetes duration. A handful of studies from India have confirmed the association of HbA1c with prevalent diabetic complications [97-99] as well as with cardiovascular disease [100].

Diabetes poses a great economic burden on the nation. A study describes a very important fact that even if the prevalence of diabetes in economically poor sections of the urban population is lower than in the high-income group, but owing to a lack of good glycemic control, the occurrence of vascular complications is higher in the former group [101]. The economic burden of diabetes in India is also enormous, and studies shows that on average, INR 10,000 (US$227) in urban areas and INR 6260 (US$142) in rural areas is the total annual expenditure by patients on diabetes care in India [102]. What is more important is the fact that low-income groups spent a higher proportion of their income on diabetes care which is 34% and 27% for urban poor versus rural poor, respectively [102]. So, diabetes should be curbed at the outset by proper monitoring and treatment. The majority of our clinicians still relay on FPG for diagnosis and monitoring of diabetes. It is to be noted here that compliance is poor in case of FPG, many laboratories in India measures serum glucose which is not reliable and consequently there is every chance of either under or over diagnosis, and moreover glucose test does not come for free although cost incurred is less. The under diagnosed cases may results in overt complications later which impose greater management cost. So if we consider these facts HbA1c though a costly affair may seem reasonable in Indian setting.

HbA1c poses all the shortcomings as already discussed. Besides India is a country where condition affecting red cell turnover like chronic malaria, hemolytic anemia, thallassemia, iron or Vit B12 deficiency anemia etc., prevails. Also sufficient data on whether Indians are high glycators or low glycators are not available [60]. But, few recent studies have presented certain data regarding HbA1c values for Indians. Studies shows that Indians have higher mean HbA1c than Chinese and Malaya [103]. Also Diabetic Care – Asia 1998 study conducted in 12 Asian nations, reports that Indian have a higher mean HbA1c [104]. Further in a study conducted by Manisha et. al., the HbA1c cut off in newly diagnosed diabetic in north and south India is found to be ≥ 5.8% which is much lower than the proposed by the international expert committee (≥6.5

%). They also depicts that a value of ≥ 5.5% or ≥ 5.6% defines IFG in Indian setting [105]. In this context we can at least assume that HbA1c cut off for Indian will vary from the ADA recommendation and though some study have provided some preliminary data, a pan Indian study suggesting the variation of HbA1c among the diverse ethnicity in India is to be conducted.

Standardization of HbA1c test in accordance with NGSP / IFCC criteria across the Indian laboratories is also matter of concern. This can be implemented only by proper awareness among treating physician and proper protocol of diabetic diagnosis and management across India.

8. CONCLUSION

HbA1c is undoubtedly a user friendly and stable test with very minimal biological variability and which is not affected by factors which otherwise has considerable impact on glucose measurement. So the compliance of diabetic subjects is increased which is an important and welcome feature in diabetic management for patient as well as the treating physicians. Before there was standardization of HbA1c methods diabetic patient failed to comply with regular FPG, OGTT or self monitoring of blood glucose (SMBG), which in turn presented difficulties in monitoring glucose load by the treating physicians. HbA1c has interfering factors, but as these factors can easily be nullified or minimized HbA1c has undoubtedly been categorized as the standard test in the prognosis as well as the diagnosis of diabetes, although the conventional glucose monitoring is used as an adjunct in most of the cases. For a developing country like India where an economic changes resulting changes in the lifestyle is posing an ever increasing load of diabetic population, a regular monitoring of mean glucose is very much in need. Moreover it is necessary when various studies have already proved that Indians developed diabetes and its complication at an early age, and diabetes as such pose an immense economic burden on the patient, family and the nation at large. HbA1c is for the moment an expensive test, but considering the load of diabetes in the country and its resultant economic burden early diagnosis and regular monitoring in order to curtail any resultant complication is a necessity. When such is the case the resultant cost of HbA1c testing can be very much justified. Moreover proper regulation regarding standardization of the methods for HbA1c should be implemented and research evaluating the diagnostic and monitoring HbA1c levels in India should be conducted, so that a countrywide range for HbA1c could be established. A wide and proper use of HbA1c is also likely to decrease the cost factor and HbA1c could become an affordable routine test in the long run.

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