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Laboratory Diagnosis of HbA1c: A Review

Abstract

The percentage of glycosylated hemoglobin A1c (% HbA1c) in human whole blood indicates the average plasma glucose concentration over a prolonged period of time and is used to diagnose diabetes. Currently, common laboratory methods to recognize glycated proteins are high-performance liquid chromatography, immunoassay and electrophoresis. The accuracy and the precision of A1c assays at least match those of glucose assays. Consequently, American Diabetes Association, the European Association for the Study of Diabetes and the International Diabetes Federation decided that the A1c assay should be recognized as the primary method for diagnosing diabetes. The recent availability of rapid, reliable, and easy-to-perform tests for detecting HbA1c has introduced rapid Diabetes diagnosis. This review thus summarizes the current information on the present and future aspects of diagnostic methods for HbA1c.

Keywords: Glycated hemoglobin (HbA1c); Enzymatic methods; Diabetes mellitus

Review Article

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Introduction

Glycated haemoglobin (hemoglobin A1c, HbA1c, A1C, or Hb1c; is also known as HbA1c or HGBA1c) is a form of hemoglobin which is measured primarily to identify the average plasma glucose concentration over prolonged periods. It is being observed that it is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. HbA1c is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin [1,2]. HbA1c is defined as haemoglobin which is irreversibly glycated at one or both N-terminal valines of the beta chains [3]. HbA1c has been the mostly used and accepted test for monitoring the glycaemic control in individuals with diabetes. Once a haemoglobin molecule is glycated, it continues to remain in the red blood cell for the rest of its life-span (120 days).

HbA1c laboratory tests are used to check control in diabetes mellitus. Haemoglobin A1 and haemoglobin A1c Chromatography of normal adult blood divides in two parts: HbA (HbA0) 92-94%. HbA1 (6-8%) in which the B chain has an additional glucose group. HbA1 consists of three different glycations, the HbA1c usually measured by isoelectric focusing or electrophoresis [4]. The glycation of haemoglobin occurs at a variable (non-linear rate) over time, during the lifespan of the red blood cell (RBC), which is of 120 days. The relative proportion of HbA1c depends on the mean glucose level over the previous 120 days. Laboratory normal range is differ depending on whether HbA1 or HbA1c is measured and on the method used [5].

HbA1c is a reliable indicator of diabetic control except in the following situations: Situations where the average RBC lifespan is significantly <120 days will usually give rise to low HbA1c results because 50% of glycation occurs in 90-120 days [6]. Common causes include:

a. Increase in red cell turnover: blood loss, haemolysis, haemoglobinopathies and red cell disorders, myelodysplastic disease.

b. Interference with the test (this depends on the method used: persistent fetal haemoglobin and haemoglobin variants, carbamylated haemoglobin (uraemic patients).

c. In patients who fluctuate between very high and very low levels - glycated haemoglobin in that case readings can be misleading (the clinician should compare with extra information obtained from home capillary blood glucose tests).

d. HbA1c can be useful in identifying patients who may be presenting an unrealistically good report of their home glucose tests.

Historical Perspective

In 1955, researchers for the first time described, that adult haemoglobin contains heterogenous molecules. By the mid 1970s, the nature of the chemical reaction had been explained. Glycation, is a spontaneous non-enzymatic reaction in which glucose binds covalently with haemoglobin at amino terminus of the b-globin chain. It is being further suggested that second carbon atom in glucose molecule was tritiated instead of the first. So in the red blood cell, glucose forms an aldimine linkage with NH_2- of valine of the β-chain, undergoes an Amadori rearrangement which forms stable ketoamine linkage as shown in Figure 1. In 1976, HbA1c was described as a useful mean for monitoring the glycaemic control in diabetic patients [7]. By the early 1980s, The HbA1c test

was widely accepted in clinical practice.

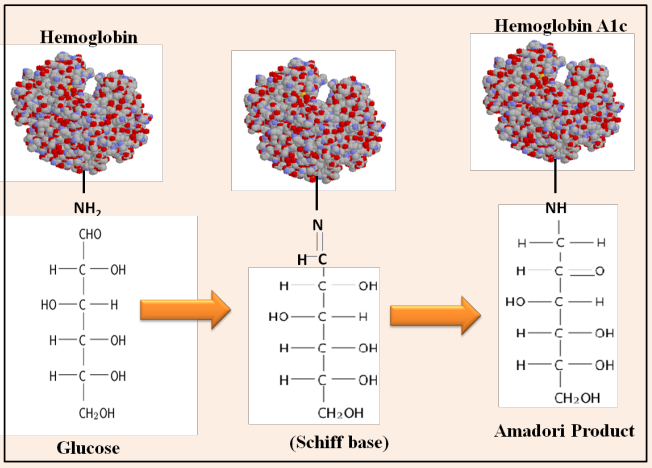

Figure 1: Chemical reaction involved in glycation of hemoglobin.

Clinical uses of HbA1c

More than 220 million people worldwide have been diagnosed with diabetes, although the actual number of people with diabetes is likely to be higher because of the insidious onset of Type 2 diabetes. Many people who have impaired glucose tolerance remain outside the diagnosed community of patients. The increase in life expectancy combined with the emergence of T2DM (Type 2 diabetes Mellitus) in children has resulted in phenomenal increase in diabetes related complications include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise, has become one of the major causes of disability and death worldwide. Type 2 diabetes accounts for 90% to 95% of all cases of diabetes. After that, T2DM increases the risk of heart disease and stroke; indeed, 50% of people with diabetes die of cardiovascular diseases [8].

HbA1c is accepted as the best measure of glycemia over the prior 3 months. There are many ways to check glycemia (e.g., history of overt symptoms [polyuria, polydypsia, etc.], urine glucose, random or fasting plasma glucose). The occasional laboratory blood glucose is the most frequently used of these assessment tools, and may be reasonably reflective of mean glycemia in stable type 2 diabetes, but it is a correct measure only of blood glucose at that moment in time. The most reliable assays of HbA1c are those performed in a high quality clinical labs, one standardized to the National Glycohemoglobin Standardization Program (NGSP) [9]. The main advantages of point-of-care testing include the fact that clinicians can know results immediately, as they see patients, rather than at some time after the visit, and the fact that point-of-care tests can be used at sites without easy access to clinical labs. The disadvantages of point-of-care testing include the need to have the reagents which should be stored properly and the possible loss of quality control when untrained personnel perform the assay. Another disadvantage, which applies particularly to home testing of HbA1c by patients, is the fact that the data do not always accurately and completely enter into electronic medical records. Disadvantages aside, there is evidence that point-of-care testing is effective [10,11].

Non-enzymatic glycation verses enzymatic deglycation

Most proteins (including haemoglobin) react with sugars to form covalent compounds without the involvement of enzymes. This chemical process is termed non-enzymatic glycation. The resulting accumulation of advanced glycation end products is associated with the progression of the complications of diabetes whereas enzymatic deglycation reverses the process of non-enzymatic glycation and generates free amino groups [12]. Enzymatic deglycation is a formidable defence system against non-enzymatic glycation in mammalian cells. This system operates using fructosamine-3-kinase (FN3K), phosphorylating fructoselysine residue on glycated proteins and thereby destabilizing the compound, ultimately causing the decomposition of the glycated proteins [13,14]. This process of enzymatic deglycation is overwhelmed by episodes of extreme hyperglycaemia in individuals with diabetes as non-enzymatic glycation continues unabated [15]. In the long run, it alters the stability of the protein structure, ultimately leading to cellular dysfunction [16].

These Advanced Glycation End products (AGEs) directly and indirectly (via receptors) promote the development of cardiovascular disease [17]. They accumulate in different parts of the body and interact with receptors for advanced glycation end products (RAGE), induce oxidative stress, increase inflammation and enhance extracellular matrix deposition, thereby accelerating the process of endothelial dysfunction. Consequently, they result in accelerated plaque formation and ultimate atherosclerosis in diabetes [18]. Glycated haemoglobin, intermediary compound is reversible but after some internal rearrangement of the compound, a stable HbA1c is formed [19]. Several glycation sites of the HbA molecule exist; N-terminal valine residue of the b-chain is the predominant glycation site, accounting for 60% of bound glucose. Of the three types of HbA1 namely, HbA1a, HbA1b, and HbA1c. HhA1c represents the most prevalent glycated species.

Standardization of HbA1c measurement; why it is necessary?

Lack of standardization resulted in wide variability within results (4.0% to 8.1%) on the same sample [20] making it difficult to compare patients results among laboratories. This disparity has always been a source of anxiety among health care providers. It becomes even more important in this age of heavy economical migration, when people travel long distances and take their native record with them. Therefore, having same method and unit to measure HbA1c is need of the day.

To overcome this problem, in 1995 the International Federation of Clinical Chemistry (IFCC) took the lead in developing a uniform international standardization of HbA1c. For the calibration of the reference method, mixtures made of pure HbA1c and HbAo were developed. A laboratory network was also setup, which use two reference assays that combined reverse-phase high performance liquid chromatography (HPLC) with mass spectroscopy or capillary electrophoresis, using same mixture as calibrators. The IFCC then defined HbA1c as haemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta- chains

[21]. This definition also covers Hb that is additionally glycated at any lysine residue in the b-chain. Prior to the IFCC's definition, HbA1c had been defined as a certain peak in an HPLC system, which obviously did not sound very scientific. Haemoglobin that is only glycated at a lysine site is not included in the measurement of HbA1c. Since the IFCC measurement is too specific, it only measures one molecular species of HbA1c: thus, non- HbA1c components are not included in final results. Consequently, HbA1c values obtained by using IFCC method are 1.5 to 2 percentage points lower than the NGSP results traced to DCCT, as well as Swedish and Japanese designated comparison methods [22].

Concerns were raised about the impact of this value change on patient care, which could result in less than desirable control of glycaemia in diabetic patients [23]. To overcome this problem a "master equation" was developed to formulize the relationship between the IFCC reference method and all three designated comparison methods (DCMs) namely, the National Glycohemoglobin Standardization program of US (NGSP), Japanese Diabetes Society/Japanese Society of Clinical Chemistry (JDS/JSCC), and Mono-S in Sweden [24]. The master equation allows for the conversion of the IFCC results to more customary HbA1c results, which could be traced to results from DCCT and United Kingdom Prospective Diabetes Study (UKPDS). In 2004, the American Diabetes Association, European Association for the study of Diabetes, and International Diabetes Federation working group of the HbA1c assay was established to harmonize the reporting systems. It included members from the ADA, IDF, EASD, NGSP and IFCC. In 2007, the IFCC recommended that HbA1c results be expressed as mmol HbA1c /mol Hb instead of an HbA1c percentage. Patients using mmol/l or mg/dl for self-monitoring of day-to-day glucose control find it difficult to understand when their doctors discussed haemoglobin levels in percentages.

To eliminate confusion and streamline these discrepancies, a consensus statement [25] on the worldwide standardization of haemoglobin A1c measurement was adopted in May 2007 by the ADA, EASD, IDF and IFCC. It states that new IFCC reference system is the only valid anchor for implementing the standardization of the measurement of HbA1c. In addition, HbA1c results were to be reported worldwide in IFCC units (mmol glycated Hb / mol total Hb) and derived NGSP units (%), using the IFCC-NGSP master equation. Thus, the 25 to 42 (mmol/mol) range would indicate non-diabetics, as the similarly derived NGSP units of the non-diabetic range were 2.5 to 4.2% (HbA1c). It was also resolved that if the ongoing "average plasma glucose study" was concluded successfully (i.e. confirmed the relationship between average blood glucose and HbA1c) then the A1c-Derived Average Glucose Equivalent would also be reported as an interpretation of HbA1c results [25].

Relationship between mean blood glucose and HbA1c

Attempts to define a true relationship between average plasma glucose and HbA1c level have been made for some times, but studies had limited utility due to fewer measurements of glucose values and the limited number of participants involved. This method is error prone, with no night time samples collected, therefore, not a true representative of 24 hour glycaemia. Nathan et al. used continuous glucose monitoring, which measures interstitial glucose levels every 5 minutes, for 3 months in both non-diabetics and diabetics with relatively stable glycaemia. They reported a mathematical relationship between HbA1c and mean blood glucose, meaning HbA1c could be expressed in an equivalent mean glucose level (i.e., in the same units as patients' self-monitoring units) [26]. However, this study is limited due to extremely small sample pool. A retrospective analysis of data from DCCT also identified a linear correlation between HbA1c and average blood glucose; however, the study population consisted of T1DM only, and DCCT was not designed to determine such a relationship [27].

A New Term to Replace HbA1c

The A1c-derived average glucose study [28] was conducted in 10 different locations in North America, Europe, and Africa. The two largest countries namely, India and China with huge diabetes population were left out, leaving it less representative. The study population comprised of 507 patients, 268 T1DM and 159 T2DM patients, and 80 non-diabetic subjects. The researchers sought to examine the relationship of average blood glucose with HbA1c across a wide range; (i.e. between HbA1c 5% to 13%). They collected approximately 2,700 blood glucose readings from each participant over 3 months period, the highest number of blood glucose readings per person to date in a single study. The goal of the study was to report glycated haemoglobin results not in the usual HbA1c percentage format but as A1c-derived averages in the same units used in self-monitoring, (i.e., mg/dl or mmol/l). The study concluded that the estimated average glucose (eAG) can now be calculated from HbA1c using a linear regression equation. This eAG will now be used to monitor glycaemia in diabetic patients as the estimated glomerular filtration rate (eGFR), which is used to monitor chronic kidney disease, from the measurement of serum creatinine.

Targets for HbA1c in treating diabetes

In clinical practice, targets for HbA1c are advised by official organizations [29,30] and guidelines suggest either <6.5% or <7.0%, with a number of caveats. Either of those levels of HbA1c signals a low risk of developing progressive microvascular complications. There is only a small difference in risk status between long-term control at the level of 6.5% or 7.0%, but the individualization of targets can make a considerable difference. In the elderly patient with multiple comorbid conditions, glycemic control has little advantage [31,32]. It also makes clinical sense to relax glycemic control for people with hypoglycemia unawareness or a past of severe hypoglycemia. A younger and stable person with diabetes and good self-care may be able to achieve better glycemic control.

How is HbA1c measured?

A chemical (electrical) charge is present on the molecule of HbA1c, and the amount of the charge differs from the charges on the different components of hemoglobin [33-35]. The molecule of HbA1c has difference in size from the other components. HbA1c may be separated by charge and size from the other hemoglobin A components in blood by a procedure known as high pressure (or performance) liquid chromatography (HPLC). HPLC which separates mixtures (for example, blood) into its various components by adding the mixtures to special liquids and passing

them under pressure through columns filled with a material that separates the mixture into its different component molecules. Because HbA1c is not affected by short-term fluctuations in blood glucose concentrations, example due to meals, blood can be drawn for HbA1c testing without regard to when food was eaten.

There are 3 major HbA1c testing methods currently available to clinical laboratories.

a. Chromatography based HPLC assay

b. Antibody based immunoassay

c. Enzyme based enzymatic assay

Chromatographic method

HPLC

The chromatographic assay uses an HPLC instrument and ion exchange or affinity column to separate HbA1c molecules from another hemoglobin molecules [36,37]. The HbA1c content is measured which is based on the ratio of HbA1c peak area to the total hemoglobin peak areas.

Boronate affinity chromatography: It is based on use of a "biological interaction" for the separation and analysis of specific analytes within a sample. For HbA1c, boronate affinity chromatography is a glycation specific method based on boronate binding to the unique cis-diol configuration formed by stable glucose attachments to Hb. This method thus measures all four stable species, altogether. The combined measure of only the four stable species has been referred to as "Total HbA1c " or by some as "True HbA1c ". Since only two fractions are present in these methods (glycated and non-glycated), the glycated portion is compared to the total and results are expressed as % HbA1c. The linearity range for the HbA1c detection is 5.3% to 17%.

Latex enhanced immunoassay method: The latex enhanced immunoassay for HbA1c is based on the interactions between antigen molecules (HbA1c) and HbA1c specific antibodies that is coated on latex beads [38,39]. This crosslinking reaction results in changes in the solution turbidity and is proportional to the amount of the antigen in the samples as depicted in Figure 2. It is found to be linear in the HbA1c range of 2.0% - 16.0 %.

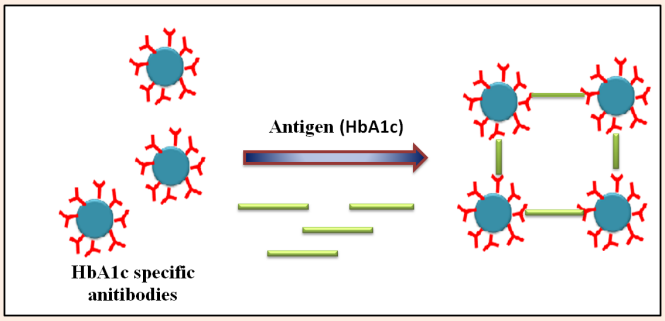

Figure 2: Direct enzymatic HbA1c method.

Enzymatic HbA1c assay method: Recent innovation has yielded a Direct Enzymatic HbA1c Assay TM which uses a single channel test and reports %HbA1c values directly, without the need for a separate THb test or a calculation step [40,41].

Assay Principle

Oxidizing agents in the lysis buffer react with the blood sample to discard low molecular weight and high molecular weight signal interfering substances. After lysis, the whole blood samples are subjected to proteolytic digestion. This process releases amino acids, including glycated valines, from the hemoglobin beta chains. The Direct Enzymatic HbA1c Assay™ glycated valines serves as substrates for a specific recombinant fructosyl valine oxidase (FVO) enzyme. The recombinant FVO specifically cleaves N-terminal valines and then produces hydrogen peroxide in the presence of selective agents. This is measured using a horseradish peroxidase (POD) catalyzed reaction and a suitable chromagen. The signal produced in the reaction is used to directly report the percentage HbA1c of the sample using a suitable linear calibration curve expressed in %HbA1c. The Direct Enzymatic HbA1c Assay principle is depicted in Figure 3.

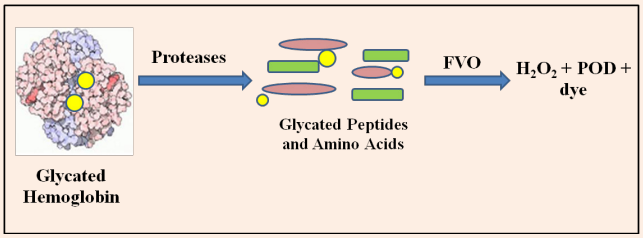

Figure 3: Latex enhanced immunoassay method.

The Direct Enzymatic HbA1c Assay™ has all the advantages of both the HPLC and immunoassays methods in accuracy, specificity, applicability to chemistry analyzers and yet is cost effective, simpler and has less interferences. The direct enzymatic HbA1c test uses 2 ready-to-use liquid stable reagents [34]. Since it does not require a separate measurement of total hemoglobin content in the samples, the Direct Enzymatic HbA1c Assay™ only needs a single channel to perform the test on chemistry analyzers in comparison with some immunoassays that require a separate measurement of total hemoglobin and need two channels for the test on chemistry analyzers.

The Direct Enzymatic HbA1c Assay™ procedure is simple and straight forward. After addition of Reagent R1, sample and Reagent R2, the result of %HbA1c will be reported within 2 min as. In addition, the reagents do not contain latex particles, and hence do not coat analyzer cuvettes and lines. Most importantly, enzymatic HbA1c assays have the highest specificity among all HbA1c assays. The direct enzymatic HbA1c method has an assay linearity range from 4 to 16%.

As mention in Table 1, enzymatic HbA1c assays are not interfered by either chemical or genetically modified hemoglobin variants. Therefore, enzymatic Hb1c tests are reliable tests, and it

does not report false results regardless of the patient's hemoglobin variant types. In summary, the Direct Enzymatic HbA1c Assay™ offers the following advantages over HPLC and Immunoassays:

a. Two reagents, liquid stable
b. No need for total hemoglobin measurement
c. Single channel on analyzers Faster, simpler and more cost effective
d. No interferences from hemoglobin variants
e. On-board blood lysis possible
f. Applicable to most analyzers
g. Excellent correlation with HPLC and immunoassays

Capillary electrophoresis

Basically, two possibilities exist for separation of HbA1c in capillary electrophoresis (CE) according to charge-to-mass ratio. Firstly analysis as cations in acidic buffers of pH below pI of Hb, which is approximately 7.0. Separation of hemoglobins A1C and A0 occurs due to a charge difference coming from elimination of one positively charged amino group in the HbA1c molecule by attachment of glucose moiety. Secondly, Hb analysis as anions in alkaline conditions with selectivity to HbA1c induced by a cis-diol interaction of its glucose unit with a borate anion from background electrolyte (BGE) (Table 1).

Table 1: Methods of testing: their advantages and disadvantages.

Method of Testing	Procedure	Advantages	Drawbacks
Chromatography based HPLC assay	a. Assay uses an HPLC instrument and ion exchange or affinity column to separate HbA1c molecules from another hemoglobin molecules. b. Based on the ratio of HbA1c peak area to the total hemoglobin peak areas.	HbA1c overestimation leads to aggressive glucose management, resulting in more frequent hypoglycaemic episodes [36].	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 [37].
Antibody based immunoassay	a. A typical method 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 [38]. b. The agglutinator reacts with the antibody to give a scattering of light and an increase in absorbance. c. 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 [38].	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 [41].	a. Enzymatic assay proved to be a robust and reliable method for HbA1c measurement suitable for routine practice in clinical chemistry laboratories [39]. 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 [40].	A disadvantage of the enzymatic method is its relatively high cost.

Electrochemical biosensor for glycated hemoglobin (HbA1c)

The first successfully commercialized biosensors were based on electrochemical sensors for multiple analytes. Studies on electrochemical biosensors had been going on for a long time. Currently, transducers based on semiconductors and screen printed electrodes shows a typical platform for the development of biosensors. Enzymes or enzyme labeled antibodies are the most used biorecognition components of biosensors [35]. Bioelectroanalysis with electrochemical biosensors is a new field in fast development within electroanalysis. In the development of biosensor, bioreceptor molecule, immobilization method and transducer should be selected properly and should be suitable for the desired work. Bioelectroanalytical sensors provide the analysis of with specificity, rapid technique, sensitive, selective and cheap in cost. The difference between biosensor and physical or chemical sensors is that its recognition element is biological [35]. Electrochemical biosensors have advantages that they can sense materials without damaging the system. Electrochemical biosensors [42-70], for the purpose of calculating daily glucose levels to control food intake and insulin usage, these glucose meters work although some difficulties exist. For example, blood glucose level measurements are recommended three to four times in a day. Due to the large fluctuations in glucose levels that naturally occurs over the course of a day, measurements on an empty stomach and within 2 hour of eating are required for differentiating purposes. These problems are more prominent for the diagnosis of diabetes and checking the link between lifestyle and medication once a patient has been diagnosed with this disease. There are various studies for detection of glycated hemoglobin which has already been done is summarized in Table 2.

Also, there are various nanomaterials like gold nanoparticles, carbon nanotubes (CNT), Core-shell magnetic bionanoparticles, Nitrogen doped grapheme etc [71-77], which can be used in constructing an electrochemical sensor as well as other different type of biosensors for example microfluidic, optical for the detection of glycated hemoglobin. So a comparison of nanomaterials based sensing devices for the detection of HbA1c has also been briefed in Table 3. These sensors exhibited linear responses to HbA1c levels of 2.5%-15%. In the present market scenario laboratory methods (Chromatography based HPLC assay, antibody based immunoassay and enzyme based enzymatic assay) costs in the range of 700 INR to 1400 INR. By using nanoparticles and its multiple usability will reduce the cost of the product over the long term then current diagnostic systems.

Table 2: Various methods for detection of glycated hemoglobin.

Year	Title	Patent No	Original Assignee	Reference
1981	Reagent and test kit for determining glycosylated hemoglobin	US 4255385 A	Abbott Laboratories	[42]
1993	Determination of glycated hemoglobin by fluorescence quenching	WO 1993018407 A1	Abbott Lab	[43]
1994	Rapid determination of glycated hemoglobin	EP 0590047 A1	Abbott Laboratories	[44]
1994	Combined glycated hemoglobin and immunoturbidometric glycated albumin assay from whole blood lysate	US 5284777 A	Isolab, Inc.	[45]
1995	Determination of glycated hemoglobin by fluorescence quenching	US5478754 A	Abbot Laboratories	[46]
1996	Method for preparing a glycated hemoglobin solution	US 5589393 A	Abbott Laboratories	[47]
1997	Methods and reagents for the rapid determination of glycated hemoglobin	US 5686316 A	Abbott Laboratories	[48]
1999	Measurement of glycated hemoglobin	WO 1999022242 A2	Abbott Lab	[49]
2000	Determination of % glycated hemoglobin	US6162645 A	Abbott Laboratories	[50]
2001	Measurement of glycated hemoglobin	US6174734 B1	Abbott Laboratories	[51]
2003	Method for quantitative determination of glycated hemoglobin	US 6562581 B2	Portascience	[52]
2004	Method for measurement of glycated hemoglobin by a rapid strip test procedure	US 6677158 B2	Exocell Inc.	[53]
2006	Method for the determination of glycated hemoglobin	US 7005273 B2	Therasense, Inc.	[54]
2008	Cellular controls for glycated hemoglobin Hb A1c	US 7361513 B2	Streck, Inc.	[55]

2008	Determination of glycated hemoglobin by fluorescence quenching	CA 2102417 C	BiBTeX, EndNote, RefMan	[56]
2009	Anti-glycated hemoglobin pan-specific monoclonal antibody	EP 1414860 B1	Dako Denmark A/S	[57]
2009	Methods for the detection of glycated hemoglobin	WO 2009067421 A1	Siemens Heathcare Diagnostics, Eddy Chapoteau, Richard Edwards, Chester Swirski, Wolodymyr Zazulak	[58]
2011	Method of measuring glycated hemoglobin concentration	US 8021887 B2	Arkray, Inc.	[59]
2012	Methods for assaying percentage of glycated hemoglobin	US 8318501 B2	General Atomics	[60]
2012	Device for the determination of glycated hemoglobin	US 8206563 B2	Abbott Diabetes Care Inc.	[61]
2012	Method of measuring glycated hemoglobin concentration and concentration measuring apparatus	US8268625 B2	Arkray, Inc.	[62]
2013	Cis di-ahl modified controls for glycated hemoglobin S-A1c derived from healthy blood cells	US 8551784 B2	Streck, Inc.	[63]
2013	Method for measuring glycated hemoglobin	US 8557590 B2	Infopia Co., Ltd.	[64]
2013	Methods for assaying percentage of glycated hemoglobin	EP 2044444 B1	General Atomics	[65]
2013	Method of preparing controls for glycated hemoglobin S-A1c derived from healthy blood cells	US 8546144 B2	Streck, Inc.	[66]
2014	Low cost electrochemical disposable sensor for measuring glycated hemoglobin	US 8702931 B2	Indian Institute Of Science	[67]
2014	Methods for the detection of glycated hemoglobin	US 8715942 B2	Siemens Healthcare Diagnostics Inc.	[68]
2014	Systems and methods for determining the percentage of glycated hemoglobin	US 20140186862 A1	Relia Diagnostic Systems, Inc.	[69]
2014	Reaction cassette for measuring the concentration of glycated hemoglobin and measuring method thereof	US 8846380 B2	Infopia Co., Ltd.	[70]

Table 3: A comparison of nanomaterials based sensing devices for the detection of HbA1c.

Serial No.	Nanomaterial	Electrode/ Method Based on	Type of Biosensor	Reference
1	Poly(terthiophene benzoic acid) (pTTBA)/ gold nanoparticles (AuNPs)	coated-screen printing electrode	Amperometric	[71]
2	Upconversion nanoparticles (UCNPs), such as NaYF4: Yb3+, Er3+	Luminescence resonance energy transfer (LRET)	Optical	[72]
3	Nitrogen-doped graphene nanosheets	Fluorine-doped tin oxide glass electrode	Electrochemical	[73]
4	Reduced Graphene Oxide	Etched fiber Bragg gratings (eFBG)	Optical	[74]
5	Self-assembled monolayers of 3-Mercaptopropionic acid (MPA)	Gold Electrode	Electrochemical	[75]
6	CNT	Based on flow injection and screen-printed electrode	Electrochemical	[76]
7	Poly(amidoamine) G4 dendrimer	Boronic acid-modified electrodes	Electrochemical	[77]
8	AuNPs	Microfluidic chip	Microfluidic	[78]

9	Biomaterials, like glycated haemoglobin, into the sensing surface	Two-dimensional photonic crystal-based biosensor	Optical	[79]
10	Core-shell magnetic bionanoparticles	Modified gold electrode	Amperometric	[80]
11	Magnetic beads	Microfluidic chip	Microfluidic	[81]

Future Implications: the challenges and solutions

HbA1c detection methods could be divided into laboratory instruments and point-of-care testing (POCT) instruments. The analytic performance of laboratory instruments is better than the performance of POCT instruments, but POCT instruments have the advantage of producing results during the patient's visit to the physician (thus meeting the clinical requirement of convenience). The development of POCT instrument is a recent trend. The ultimate challenge is to find an analytic device with good specificity and clinically relevant imprecision. The development of cheap and disposable array biosensors for the simultaneous detection of clinically important diabetic markers is still needed. The use of biomolecules to grow NPs has great promise in the future of biosensing and design of bioelectronic systems.

Conclusion

Daily self blood testing, measured in mmol/L or mg/dl and HbA1c measurement in percentage are somewhat confusing. Given the narrow range of percentages, it is sometimes difficult for patients to comprehend the consequences of even a 1 percent increase or decline in HbA1c. Patients and their caretakers are used to the idea that the HbA1c level should be less than 7% in diabetic patients: a higher reading indicates that the glycaemic control is getting out of hand. Now the IFCC results will be provided in mmol HbA1c per mol haemoglobin. Keeping the NGSP results in percentages along with IFCC results will make the change less confusing.

Our objective was to compare in a prospective study the clinical performance of the reference and an alternative method to measure blood levels of glycated hemoglobin. The rapid and accurate laboratory diagnosis of HbA1c is necessary through a variety of laboratory modalities. Such a testing is done so far by HPLC, immunoassay, enzymatic reactions but all have limitations. An HbA1c biosensor may be a better option due to low cost, rapidity, and high sensitivity factors.

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