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Glycated Haemoglobin Levels and Type 2 Diabetes Mellitus

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KEYWORDS

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ABSTRACT:

Prevalence of diabetes, especially type 2, has reached epidemic levels worldwide. HbA1c is one of the best tests for diagnosing and monitoring diabetes. HbA1c measures average blood sugar levels over 2-3 months by looking at glycated haemoglobin levels. It helps set treatment goals to reduce diabetes complications. However, there are many factors that can cause variation and inaccuracy in HbA1c results, such as anaemia, certain medications, HIV/hepatitis infections, and haemoglobin variants. Over 700 haemoglobin variants can interfere with HbA1c measurement, so test methods like HPLC may need modification for accurate results. While accurate and recommended by diabetes associations for diagnosis, HbA1c has mixed acceptance worldwide as a sole diagnostic test. In India, lack of standardization, prevalence of haemoglobin disorders, and fragmented healthcare access limit using HbA1c alone for diagnosis. Supporting tests are often needed. The review concludes that in the current Indian scenario, HbA1c cannot be the sole independent test for diagnosing diabetes due to various limitations.

1. Introduction

1.1 Background

Over 220 million individuals have been diagnosed with diabetes globally, but because Type 2 diabetes has a insidious onset, the true number of diabetics is probably greater. Many persons with reduced glucose tolerance do not belong to the group of patients who have been diagnosed. A significant rise in diabetes-related complications, such as smoking, high blood pressure, obesity, elevated cholesterol, and irregular exercise, has been brought about by an increase in life expectancy and the emergence of Type 2 diabetes Mellitus (T2DM) in children. Diabetes is now one of the leading causes of disability and mortality globally. Between 90% and 95% of instances of diabetes are type 2 diabetes. A spike in blood glucose level known as "hyperglycaemia" is a hallmark of diabetes mellitus, a chronic metabolic illness [1]. Over the past 20 years, the high prevalence of diabetes mellitus has become a global public health concern. Approximately 85–90% of cases of diabetes are thought to be type 2 [2]. Type 2 diabetes is the most prevalent kind of the disease. By 2030, the global prevalence of diabetes is predicted to increase from 2.8% in 2000 to 4.4%. This corresponds to a predicted increase in the number of people with diabetes from 171 million in 2000 to well over 350 million in 2030. There are two forms of diabetes: type 1 affects 5% of the population, and type 2 affects 95% of diabetics. This necessitates better management of hyperglycaemia and additional metabolic syndrome risk factors [3]. Impaired glucose tolerance (IGT) is a risk factor for macrovascular disease and a predictor of the later onset of Type 2 diabetes. In its early stages, type 2 diabetes frequently has no symptoms and can go undiagnosed for years [4]. Given that the risk of both micro and macrovascular problems can be significantly reduced [3]. Continuously high blood sugar levels raise the risk of long-term vascular complications from diabetes, including gangrene, gastroparesis (slowed stomach emptying), neuropathy (loss of sensation, especially in the feet), coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, and erectile dysfunction. Inadequate management of blood glucose also raises the possibility

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of transient surgical problems, such as delayed wound healing. The best screening test to identify diabetes is not widely agreed upon. The oral glucose tolerance test (OGTT) and the fasting plasma glucose (FPG) test are the two most popular screening assays. Blood glucose is measured as part of these two tests. However, patients must fast for at least eight hours the night before both the OGTT and FPG measurements, and the test must be done at least twice to confirm the diagnosis when utilizing the FPG. Moreover, research indicates that the sensitivity of FPG for the diagnosis of diabetes is not as high as anticipated, with about one-third of diabetics going undiagnosed [5]. In addition to being expensive, labour-intensive, time-consuming, and having low repeatability, OGTT might cause confusion and doubt when it comes to the confirmation of diabetes diagnoses [6]. Errors in the laboratory, non-adherence to fasting by the patient, and/or usage of specific drugs can all lower the accuracy of FPG and OGTT [5]. Haemoglobin A1c, also known as glycosylated haemoglobin, HbA1c, A1C, or Hb1c; occasionally also known as HbA1c) is a type of haemoglobin that is mostly used to measure the average plasma glucose concentration over extended periods of time. It is created through a non-enzymatic process as a result of haemoglobin's regular exposure to elevated glucose plasma levels [7]. Another test for Type 2 diabetes screening that has been proposed is the glycated haemoglobin (HbA1c) test. A two- to three-month average of blood glucose concentrations is represented by HbA1c readings. One reliable way to track glycaemic management in individuals with diabetes mellitus is to assess glycosylated haemoglobin (GHb) [8]. It was first suggested in 1976 [9] to utilize haemoglobin A1c to track how well diabetes patients' glucose metabolism is under control. The Diabetes Control and Complications Trial (DCCT) for type 1 diabetes [8] and the United Kingdom Prospective Diabetes Study (UKPDS) for type 2 diabetes [10] demonstrated the clinical importance of the HbA1c test. The results of the research demonstrated the significance of HbA1c in determining a patient's risk of hypoglycaemia and microvascular problems. As a result, normal therapy of patients with type 1 and type 2 diabetes today includes assessment of both HbA1c and blood glucose levels [11]. Although laboratory error, the use of certain drugs, haemoglobinopathy, and renal insufficiency may affect the accuracy of the HbA1c analysis [5], the HbA1c test is more expedient and convenient than the OGTT. Regardless of the length of the fast or the makeup of the preceding meal, HbA1c can be measured at any time of the day. A tiny blood sample can also be used to analyse HbA1c using a portable instrument, albeit this is currently a costly option [12]. Additionally, blood drawn via a finger prick can be transferred to a central laboratory for examination, which enables screening of people in far-off places [13, 14]. OGTT, FPG, and HbA1c are comparable as indicators of the emergence of retinopathy and nephropathy [15-18].

1.2 Formation of glycated haemoglobin through glycation

Hyperglycaemia causes a reversible alteration in which there is an excessive generation of the early glycation products. These glycation products are generated both inside and outside the cells, as glucose rapidly attaches to the amino groups of the proteins by the non-enzymatic process of nucleophilic addition, to form schiff base adducts. These adducts attain equilibrium levels proportionate to blood glucose concentration in a matter of hours. They then undergo rearrangement to generate more stable early glycation products, which take many weeks to achieve equilibrium. Glycated haemoglobin is one of the proteins that is glycated in this manner [19]. An accumulation of glycated haemoglobin within a red blood cell, which occurs when a haemoglobin molecule becomes glycated, therefore represents the average amount of glucose that the cell has been exposed to during its life cycle. Monitoring long-term serum glucose management through measurement of glycated haemoglobin allows one to evaluate the efficacy of treatment. The average blood glucose concentration throughout the preceding four weeks to three months is directly correlated with the HbA1c level. According to some experts, most of its usefulness is attributed to a much shorter time frame of two to four weeks [20]. Several processes related to diabetes problems may be negatively impacted by the overproduction of early glycation products. LDL uptake may be increased in blood vessels, which could lead to atherogenesis [21] and an increase in damage caused by free radicals [22]. The pathophysiology of the early functional alterations in the diabetic microvasculature is likely influenced by these reversible biochemical abnormalities. As a result, measuring HbA1c, a measure of long-term average glycemia, helps diabetics and their doctors by establishing treatment objectives that lower the risks of

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chronic diabetes problems developing and worsening [23].

1.3 Role of non-enzymatic glycation and enzymatic deglycation

Without the aid of enzymes, the majority of proteins, including haemoglobin, combine with carbohydrates to produce covalent compounds. Non-enzymatic glycation is the name given to this chemical reaction. While enzymatic deglycation reverses the process of non-enzymatic glycation and produces free amino groups, the buildup of advanced glycation end products that results is linked to the development of diabetes problems [24]. In mammalian cells, enzymatic deglycation serves as an effective defence mechanism against non-enzymatic glycation. Fructosamine-3-kinase (FN3K) is the mechanism of action of this system; it phosphorylates the fructose lysine residue on glycated proteins, destabilizing the molecule and ultimately leading to the breakdown of the glycated proteins [25, 26]. In those with diabetes, periods of severe hyperglycaemia overpower this process of enzymatic deglycation while non-enzymatic glycation persists unchecked [27]. Over time, it modifies the protein structure's stability, which eventually results in cellular malfunction [28]. The development of cardiovascular disease is both directly and indirectly aided by these Advanced Glycation End Products (AGEs) through receptors [29]. Endothelial dysfunction is accelerated by their accumulation in many body areas, interactions with advanced glycation end product (RAGE) receptors, induction of oxidative stress, augmentation of inflammation, and enhancement of extracellular matrix deposition. As a result, they cause diabetes by hastening the production of plaque and ultimately leading to atherosclerosis [30]. Although the intermediate compound, glycated haemoglobin, can be reversed, a stable HbA1c is created once the complex undergoes some internal reorganization [31]. The HbA molecule has many glycation sites, with the N-terminal valine residue of the b-chain being the most prominent one, responsible for 60% of the bound glucose. Of the three forms of haemoglobin A1 (HbA1a, B, and C). The most common species that is glycated is represented by HhA1c [32].

2. Measurement of Hba1c

The HbA1c molecule has a chemical (electrical) charge, and the quantity of this charge varies from that of the other haemoglobin components [33–35]. The size of the HbA1c molecule differs from the other constituents. Charge and size differences between HbA1c and other haemoglobin can be seen. elements in blood using a technique called high performance (or pressure) liquid chromatography (HPLC). HPLC is a technique that breaks down mixtures (like blood) into their constituent parts by combining the mixtures with certain liquids and forcing them through columns that are packed with a substance that breaks down the combination into its individual molecule components.

Clinical laboratories today have access to three main HbA1c testing methodologies, namely

a. Chromatography based HPLC assay
b. Antibody based immunoassay
c. Enzyme based enzymatic assay

2.1 Chromatographic method HPLC

The chromatographic assay distinguishes between HbA1c and other haemoglobin components using an HPLC device and an affinity or ion exchange column [36, 37]. Based on the ratio of the HbA1c peak area to the total haemoglobin peak regions, the HbA1c content is determined. The basis of benzoate affinity chromatography is the analysis and isolation of particular analytes within a sample through the application of a "biological interaction." A glycation-specific technique for HbA1c, boronate affinity chromatography relies on boronate binding to the distinct cis-diol structure created by stable glucose attachments to Hb. Thus, in all, all four stable species are measured using this approach. Some have referred to the combined measurement of just the four stable species as "True HbA1c" or "Total HbA1c." Since these procedures only provide two fractions (glycated and non-glycated), the results are presented as a percentage of HbA1c and the glycated component is compared to the whole. 5.3% to 17% is the linearity range for HbA1c detection. The interactions between antigen molecules (HbA1c) and HbA1c specific antibodies coated on latex beads are the basis of the latex enhanced immunoassay for HbA1c [38, 39]. As shown in Figure 2, this crosslinking reaction causes variations in the turbidity of the solution that are proportionate to the antigen content of the samples. It is discovered to be linear between 2.0% and 16.0% in the HbA1c range. The Direct Enzymatic HbA1c Assay TM is a recent

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innovation that employs a single channel test and gives %HbA1c readings instantly, eliminating the need for a calculation step or separate tHb test [40, 41].

Method of Testing	Procedure
Chromatography based HPLC assay	a. An HPLC device and an affinity or ion exchange column are used in the assay to distinguish between HbA1c and other haemoglobin molecules. b. Determined by dividing the total haemoglobin peak areas by the ratio of the HbA1c peak area.
Antibody based immunoassay	a. A common technique involves the application of a particular antibody, typically a monoclonal one, to the glucose and the initial 5–10 amino acids of the β-chain. The coating on this antibody is latex [38]. b. The antibody and agglutinator combine to produce an increase in absorbance and light scattering. c. This yields the HbA1c level, and the total haemoglobin can be measured at or around the haemoglobin Sorbet absorption band (410–420 nm), by the Drabkins method (oxidation and conversion to cyanmethaemoglobin) at roughly 540 nm, or by utilizing the alkali hematin test.
Enzyme based enzymatic assay	Samples of lysed blood are digested by proteases. Fructosyl valine oxidase uses the released glycated valines as a substrate. Using a chromogen and horseradish peroxidase-catalysed reaction, the amount of hydrogen peroxide produced is quantified [41].

2.2 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 haemoglobin beta chains.

1. Direct Enzymatic HbA1c Assay: This assay uses oxidizing agents in the lysis buffer to remove interfering substances from the blood sample. Proteolytic digestion is performed to release glycated valines from haemoglobin beta chains. A recombinant fructosyl valine oxidase (FVO) enzyme specifically cleaves the glycated valines. The FVO enzyme produces hydrogen peroxide, which is measured using a horseradish peroxidase (POD) catalysed reaction with a chromagen. The signal produced is used to directly report the percentage of HbA1c in the sample using a calibration curve. This method combines the accuracy and specificity of HPLC and immunoassays while being cost-effective, simpler, and having fewer interferences. It requires only a single reagent channel on chemistry analysers [34].

2. Capillary Electrophoresis (CE) for HbA1c Separation: HbA1c can be separated based on its charge-to-mass ratio using CE. One approach is to analyse cations in acidic buffers (pH below the pI of haemoglobin, ~7.0), where HbA1c and HbA0 separate due to the charge difference caused by the glucose attachment. Another approach is to analyse anions in alkaline conditions, where HbA1c selectivity is induced by the cis-diol interaction of its glucose unit with a borate anion from the background electrolyte [35]. Electrochemical Biosensors for HbA1c: Electrochemical biosensors were among the first successfully commercialized biosensors for multiple analytes. These biosensors use enzymes or enzyme-labelled antibodies as biorecognition components. Semiconductors and screen-printed electrodes are common transducer platforms for biosensor development. Bio electroanalytical sensors provide specific, rapid, sensitive, selective, and cost-effective analysis. Electrochemical biosensors have the advantage of being able to sense materials without damaging the system. While glucose biosensors for daily glucose monitoring exist, they have limitations in accurately capturing the fluctuations in glucose levels throughout the day [42-70].

Overall, the text discusses various techniques, ranging from enzymatic assays to electrophoretic and electrochemical methods, used for the accurate and reliable measurement of HbA1c levels, which is crucial for the diagnosis and monitoring of diabetes.

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3. Clinical significance of HbA1c in diabetes monitoring

One type of haemoglobin that is measured mainly to determine the average plasma glucose concentration over extended periods of time is called glycated haemoglobin. It has been noted that exposure of haemoglobin to plasma glucose forms it through a non-enzymatic glycation process. The beta-N 1-deoxy fructosyl component of haemoglobin is measured by HbA1c [32, 71]. Haemoglobin that has undergone irreversible glycosis at one or both of the beta chains' N-terminal valines is known as HbA1c [72]. The most widely used and recognized test for tracking glycaemic management in diabetics is the HbA1c. A glycated haemoglobin molecule stays within a red blood cell for the remainder of its 120-day life span. Laboratory tests measuring haemoglobin A1c are used to assess diabetes mellitus management. The components of haemoglobin A1 and A1c Normal adult blood is divided into two sections by chromatography: HbA (HbA0) 92-94% and HbA1 (6–8%), where there is an extra glucose group in the B chain. Three distinct glycations make up HbA1, and isoelectric focusing or electrophoresis are typically used to determine HbA1c [73]. The 120-day life lifetime of a red blood cell (RBC) allows for the varied (non-linear) rate of haemoglobin glycation over time. The average blood glucose level over the preceding 120 days determines the relative percentage of HbA1c. Depending on the procedure and whether HbA1 or HbA1c is tested, the laboratory normal range varies [74]. A good measure of diabetic management is HbA1c, except for the following circumstances: Low HbA1c readings are typically the outcome of situations where the average RBC lifespan is much less than 120 days, as 50% of glycation occurs in 90-120 days [75]. Since 50% of diabetics pass away from cardiovascular illnesses, type 2 diabetes raises the risk of heart disease and stroke [76]. The most reliable indicator of glycemia throughout the previous three months is the HbA1c. There are numerous methods to measure blood sugar, including random or fasting plasma glucose, urine glucose, and a history of overt symptoms (polyuria, polydypsia, etc.). The most often utilized of these evaluation methods is the sporadic laboratory blood glucose test, which may be a good representation of mean glycemia in people with stable type 2 diabetes, although it is right estimate of blood glucose at the moment. The HbA1c assays that are conducted in top-notch clinical laboratories and that are standardized to the National Glycohemoglobin Standardization Program (NGSP) are the most trustworthy [77]. The primary benefits of point-of-care testing are its ability to be employed at locations without convenient access to clinical labs and its ability to provide data to physicians as soon as they meet patients, as opposed to sometime after the visit. The requirement for reagents, which should be stored carefully, and the potential loss of quality control when untrained workers conduct the assay are the drawbacks of point-of-care testing. Another drawback is that patient data do not always enter completely and precisely into electronic medical records. This is especially true for HbA1c testing done at home. Diabetes is characterized by persistent hyperglycaemia high enough to result in consequences unique to the disease. Compared to single measurements of glucose concentration, laboratory techniques that capture long-term glycaemic exposure should give a more accurate indicator of the existence and severity of the condition. Research has consistently shown that retinopathy and A1C have a substantial link [80–82], while there is less evidence of a relationship between the two and fasting glucose levels [83]. In the context of controlled clinical trials for type 1 [84] and type 2 [85] diabetes, the relationship between A1C levels and complications has also been demonstrated. These results have been utilized to create the generally recognized A1C treatment objectives for diabetes management [86]. A1C levels have now been linked to a rise in the prevalence of moderate retinopathy, according to a large body of data from a variety of populations. This strong evidence supports the use of an A1C cut point of >6.5% for the diagnosis of diabetes. The A1C level of 6.5% is sensitive and specific enough to identify people who should be diagnosed with diabetes and who are at risk of developing retinopathy, but it should not be interpreted as an exact boundary between normal glycemia and diabetes. The present FPG and 2HPG readings are claimed to be more predictive than the A1C level. The International Expert Committee weighed the limited clinical repercussions of delaying the diagnosis of someone with an A1C level >6.5% against the stigma and costs associated with incorrectly diagnosing people as diabetics when choosing a diagnostic A1C level >6.5%. The American Diabetes Association supports the recommendation of an International Expert Committee to utilize the A1C test to diagnose diabetes, with a

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threshold of >6.5%, following a thorough examination of both established and emerging epidemiological data. As with the diagnostic thresholds for FPG and 2-h PG, there is an inflection point for the prevalence of retinopathy associated with the diagnostic A1C cut point of 6.5%. A method certified by the National Glycohemoglobin Standardization Program (NGSP) and traceable to the Diabetes Control and Complications Trial (DCCT) reference assay should be used to perform the diagnostic test. Point-of-care Currently, A1C tests lack the necessary accuracy to be used for diagnostics.

ADA 2010 Criteria for the diagnosis of diabetes: [87]

I. 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
II. FPG >126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.* OR
III. 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
IV. In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose >200 mg/dl (11.1 mmol/l).

*In the absence of unequivocal hyperglycaemia, diagnosis requires two abnormal test results from the same sample or in two separate test, criteria I-III should be confirmed by repeat testing.

The HbA1c test is a simple, reliable procedure with instantaneous results. Diabetes can be accurately diagnosed with it, especially in low-income nations and among groups that are difficult to reach. While HbA1c is a commonly used test for diabetes diagnosis worldwide, there is continuous discussion regarding testing methodologies and cutoff points. The diagnostic accuracy of the Fasting Glucose Test (FGT) and HbA1c alone is considerably increased when both tests are combined, though. The distinct capacity of the HbA1c test to evaluate previous glycaemic control and forecast the lipid profile in diabetic patients accounts for its predictive promise. The HbA1c test may be utilized as a diagnostic and predictive tool in the global diabetes epidemic, resulting in better patient treatment and favourable clinical outcomes [88]. Patients in the Intensive Cardiac Care Unit (ICCU) often have diabetes mellitus (DM) and prediabetes (Pre-DM). A worse prognosis is associated with these patients if their HbA1c level is 5.7 g% or greater, which is suggestive of prediabetes and diabetes. Remarkably, after being admitted to the intensive care unit, individuals with prediabetes appear to have the highest risk of dying. This unexpected association between prediabetes and an elevated risk of mortality requires more investigation [89]. Evidence-based medicine approaches have led to a thorough examination of the use of HbA1c as a diabetes diagnosis tool. This data primarily focuses on predicting clinical outcomes, particularly microvascular problems, which are seen to represent the pinnacle of the Stockholm Hierarchy when it comes to reference intervals and clinical judgment. Pre-analytical, analytical, and other biological aspects, in addition to accessibility and cost, must also be taken into account. As a diagnostic test for diabetes, HbA1c clearly outperforms glucose, particularly OGTT, although there are some significant limitations that physicians should be aware of. As always, there is a requirement for teaching materials that are publicly accessible and for doctors and the laboratory to have ongoing communication [90]. The American Diabetes Association's 2014 guidelines include four techniques for diagnosing diabetes, the first of which is a level of higher than 6.5% for glycosylated haemoglobin (HbA1c). The significance, benefits, and disadvantages of utilizing HbA1c as a diabetes diagnosis tool in comparison to alternative approaches are examined in 47 US research that are summarized in this review of the literature. The use of HbA1c as a diagnostic tool for diabetes mellitus is relatively new, hence a hybrid systematic, shortened literature review was carried out. According to the study's findings, HbA1c screening is a practical and reliable way to identify diabetes. In community-based and acute care settings where fasting testing are not feasible, it is very helpful. Nonetheless, there are drawbacks to diagnosing diabetes with HbA1c. For instance, the prevalence of diabetes may be underestimated by HbA1c testing, particularly in the case of White people. Because of this racial bias, prevalence and health disparity estimates derived from HbA1c screening are not the same as those derived from other diagnostic techniques. Moreover, recent data indicates that HbA1c testing might not be appropriate for some categories, including children, women with gestational diabetes, HIV patients, and prediabetes. To make clear

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the proper application of HbA1c screening in these populations, more guidelines are required [91].

4. Correlation between blood glucose levels and HbA1c in diabetes management

There is a complicated link between plasma glucose (PG) and A1c. Individuals with diabetes mellitus or other conditions characterized by consistently high blood sugar have higher HbA1c levels. The HbA1c level of a diabetic with well-controlled blood sugar is in or near the reference range. While the American Diabetes Association (ADA) advises that the HbA1c be below 7.0% for most patients, the International Diabetes Federation and American College of Endocrinology (IDFACE) recommends HbA1c values below 6.5% [92]. An average HbA1c of 6% is equivalent to 135 mg/dl of mean plasma glucose. Mean plasma glucose rises by 35 mg/dl for every 1% increase in A1C. HbA1C in non-diabetics is typically 3.5–5.5%. A healthy 6.5% of people have diabetes. A1C is an indicator of mean plasma glucose throughout the weeks to months prior, according to numerous research. As stated, A1C does not accurately represent glycaemic management throughout the previous three months. Instead, it is skewed toward the most recent weeks. Half of the result is determined by the mean glycemia in the month before the A1C measurement, another 25% is determined by the period between 30 and 60 days before the measurement, and the remaining 25% is determined by the period between 60 and 120 days before the measurement [93]. Table 1 lists the HbA1c values that correspond to the glucose level. Equation [94] provides an approximation of the mapping between HbA1c readings and eAG (estimated average glucose) measurements. eAG(mmol/l) = 1.59 × A1C − 2.59 eAG(mg/dl) = 28.7 × A1C − 46.7 It was discovered that a borderline (5.6–6.4%) or high (≥6.5%) HbA1c level strongly predicted the course of future medication treatment for diabetes mellitus [95].

Table 1: HbA1c values corresponding to glucose level

HbA1c	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)

In 2017, An International Consensus on the Use of Continuous Glucose Monitoring [96] standardised the use of CGM and advised its combination with HbA1c to support therapy modifications in patients with type 1 (T1DM) and type 2 (T2DM) diabetes mellitus, particularly those who experience hypoglycaemia on a regular basis. The consensus also suggested that all patients should receive instruction on how to use the various tools and technologies for glycaemic control, including how to obtain, understand, and respond to inquiries about it. The relationship between the glucometer and CGM calibration, as well as the permissible mean absolute relative difference (MARD) and ISO (International Organization for Standardization) norms, were defined as basic requirements for CGM performance. The International Hypoglycaemia Study Group (IHSG) recommendations were followed by the consensus, which also considered hypoglycaemia definitions as clinical trial standardization and classified them into levels 1, 2, and 3. This was done based on a joint position statement from the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) [97]. Level 1 (between 54 and 70 mg/dL) of this Time Bellow Range (TBR) is of little significance in clinical investigations. Level 2 (less than 54 mg/dL) needs to be reported since it has significant clinical implications. When outside help is required, level 3 hypoglycaemia is deemed severe even in the absence of a specified blood glucose level. An incident is deemed hypoglycaemic if it lasts for at least fifteen minutes. After the glycemia reaches values outside of that range, 15 minutes should pass before a hypoglycaemic episode is regarded to have ended. Exposure to hyperglycaemia is defined as the proportion of time that glucose levels are greater than 180 mg/dL. Level 1 (alert level, > 180 mg/dL to < 250 mg/dL), Level 2 (clinically significant, > 250 mg/dL), and Level 3 (clinical diagnosis: ketoacidosis or hyperosmolar hyperglycaemic condition) are the three categories for hyperglycaemia (Time Above Range, or TAR). By dividing the duration of hypo- and hyperglycaemia into

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three phases, one may more assertively determine the severity and determine the best course of action.

5. Limitations of HbA1c test

The available information in the literature remains inconsistent when it comes to choosing the most appropriate diagnostic test for diabetes mellitus. While some studies have found that HbA1c is a screening test that is as useful as the oral glucose tolerance test (OGTT) and either better than or equivalent to fasting plasma glucose (FPG), other studies have found the opposite [98]. HbA1c on its own may not always provide an accurate measure of glycaemic control in certain diabetics, and treatment decisions may not be properly impacted [99]. The HbA1c score may be affected by changes in the amount of glucose that crosses the erythrocyte membrane, changes in the rate at which glycosylation occurs, or changes in the lifespan of the erythrocyte. Any illness that results in erythrocytes having a longer or shorter lifespan or that is linked to a slower or quicker rate of regeneration will cause the HbA1c score to rise or fall in proportion to the amount of time that erythrocytes are exposed to the effects of glucose [100]. Furthermore, red blood cell lifespans in healthy individuals range significantly from one another. Compared to younger red blood cells, which are more abundant and have an average half-life of roughly thirty days, older red blood cells have undergone a greater degree of glycosylation in the past. Erythrocyte glycosylation over the last 30 days contributes half of the result of a given HbA1c measurement, but red blood cell glycosylation aged 90–120 days only shows 10% of its characteristics [101].

5.1 False rise in HbA1c levels

Inauthentic rise HbA1c levels may be unintentionally raised by conditions that enhance the longevity of red blood cells, such as functional asplenia, iron deficiency anaemia, and B12 and folic acid deficiency anaemia because they inhibit erythrocyte growth or blood cell death. Notable disorders linked to erroneously increased HbA1c include severe hypertriglyceridemia (>1750 mg/dL), severe hyperbilirubinemia (>20 mg/dL), and uraemia (caused by carbaminohaemoglobin). Consuming alcohol can lead to a misleading elevation in HbA1c values because alcohol and acetaldehyde can form a complex. Furthermore, several medications and chemicals, including salicylates, opioids, and lead poisoning, have been shown to affect HbA1c levels [102].

5.2 False decline in HbA1c levels

However, several disorders, such as anaemia from blood loss, haemolytic anaemia, which causes anaemia by decreasing the lifespan of erythrocytes, and splenomegaly, which increases red cell turnover, can result in erroneously lowered levels of HbA1c [103]. The shortened life span of red blood cells during pregnancy also causes a misleading drop in HbA1c readings. Furthermore, erroneously lower HbA1c values have been linked to the use of vitamin E, ribavirin, interferon A, cephalosporins, levofloxacin, penicillin, anti-inflammatory medications, and quinine (which can cause haemolytic anaemia) [102, 103]. It is important to discuss the contentious impact of vitamin C, which is frequently found in over-the-counter vitamin supplements, on HbA1c levels, particularly in light of the COVID-19 pandemic. Diverse results have been reported from randomised clinical trials (RCTs) examining the impact of vitamin C on multiple glycaemic control indicators, including HbA1c. Vitamin C supplementation did not alter the HbA1c concentration, as shown by a meta-analysis and systematic review conducted by Ashor et al. [104]. Furthermore, a significant decrease in glucose levels was seen without a significant impact on HbA1c levels, according to the data synthesis from five RCTs of vitamin C treatment. Nevertheless, a meta-analysis that was previously published indicated that HbA1c levels had significantly decreased [105]. Furthermore, depending on the technique of measurement, HbA1c is reliable in heterozygous types of haemoglobinopathy but unreliable in homozygous patients. When the patient's daily glucose readings and the HbA1c value differ, when the HbA1c value is greater than fifteen percent, when the HbA1c value differs significantly from prior values, and when anaemia with aberrant red cell indices is present, haemoglobinopathy may be present. Falsely lower HbA1c readings have been reported in genetic persistence of HbF, either in homozygous or heterozygous form [106].

Conclusion

HbA1c levels can be falsely elevated or decreased due to various conditions that affect the lifespan or turnover of

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red blood cells, such as haemolytic anaemia, iron deficiency anaemia, or splenomegaly. Several genetic variants, like haemoglobinopathies, can interfere with the accurate measurement of HbA1c levels. Certain medications, supplements (like vitamin C), and substances like alcohol can also cause falsely high or low HbA1c readings. HbA1c may not be a reliable diagnostic test in specific populations like pregnant women, children, patients with HIV or end-stage renal disease, or those with prediabetes. While HbA1c is a convenient test, it may not provide a complete picture of glycaemic control for all patients when used alone. It should be interpreted in conjunction with other clinical factors and self-monitoring of blood glucose. In essence, HbA1c is a valuable tool for diagnosing and monitoring diabetes, but healthcare providers must be aware of its limitations and potential interfering factors to ensure accurate interpretation and appropriate clinical decision-making.

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