Revisiting Role of HbA1c

Sanjyoti Panchbudhe¹, Shilpa Kumar¹ and Suresh Babu Kondaveeti¹

¹Symbiosis Medical College for Women, Symbiosis International (Deemed University), Pune, Maharashtra, India.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i43A32492

Editor(s):
(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
(2) Abdul Nazer Ali, AIMST University, Malaysia.
(1) Hasta Handayani Idrus, Universitas Muslim Indonesia, Indonesia.

Complete Peer review History: https://www.sdiarticle4.com/review-history/72795

Received 25 June 2021
Accepted 31 August 2021
Published 06 September 2021

ABSTRACT

With the increasing use of HbA1c as a diagnostic marker, more values at the lower end of the reference range can be observed. As more emphasis has been on decreasing and controlling a rising value of glycated haemoglobin, it is currently uncertain how to interpret low HbA1c values. Various conditions not related to diabetes, but with a fairly common occurrence, influence HbA1c. Alternative indices may have to be used for assessing glycemic control in these cases. It is important that such influencing factors are looked into when evaluating an inappropriately or expectantly low HbA1c value. HbA1c is a biomarker that might have utility beyond just diabetes mellitus. Its role needs to be re-examined as it is a test which is widely available, less amenable to short term physiological variations, and can be easily studied. All that is required is a different perspective or approach to its utility.

Keywords: Diagnostic; glycemic; HbA1c; physiological variations; marker.

1. INTRODUCTION

Haemoglobin is a quaternary protein composed of four globin chains, two alphas and two betas. Hemoglobin A1c (HbA1c) is the gold standard for measuring long-term glycemic management in people with diabetes mellitus. Patients with alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) who have diabetes mellitus are at increased risk for developing...
fibrosis and fibrosis progression, according to several studies. HbA1c is a fraction of haemoglobin that has undergone a non-enzymatic attachment to glucose moiety at valine, the beta chain. Since this glycation is non-enzymatic, the presence of glucose in the vicinity is one of the major factors contributing to this glycation. The higher the concentration of glucose, the more will be the glycation. Once a glucose moiety is attached, haemoglobin remains glycated for the rest of the Red Blood Cell (RBC). Hence, glycated haemoglobin (HbA1c) gives a look into the glycemic milieu an RBC has been exposed to and thereby allows the assessment of glycemic control spanning over the past 6-8 weeks, which is the assumed half-life of an RBC. Due to the close link of HbA1c with glycemic control, it has always been known for its role, with a value of >6.5% as being diagnostic [1]. Apart from diagnosis, its role in therapeutic monitoring is also quintessential. The chronic complications in diabetes like nephropathy, neuropathy, cardiovascular disease etc., can be prevented only with continuous monitoring and prompt treatment, in which HbA1c has a crucial role to play. The importance of HbA1c is well established however, low values of HbA1c have not received the same attention or exploration.

1.1 Is the Lab to Blame?

A low HbA1c value would not usually sound like a cause of concern. Rather it is the target of many patients and their physicians, but how does one deal with values lower than the reference interval of 4%? How low is too low? This is not a very commonly encountered scenario but with the increasing and widespread use of HbA1c, it is no longer unheard of. In such cases the onus lies on the laboratories to ensure accuracy and precision of their reports. Haemoglobin variants are a common and well known to be responsible for a low HbA1c value but not all methods are capable of detecting the presence of such variants [2]. Irrespective of the limitation of the assays and variants, usually the foremost concern on seeing an unusual report in day to day practice is the presumption that it might be a laboratory error. Such errors can be ruled out on following a systematic assessment of the possible sources.

1.2 Ruling out Lab Error

1. ‘Repeat the test using fresh sample before issuing the report’ as the first line intervention and if necessary maybe recommended to follow (points 2 to 8) recalibration etc of the instrument apart from other checkpoints.

2. Consider limitations of the methodology:

   a. What is the reportable range, sensitivity and linearity?
   b. Can the method detect haemoglobin variants and at what level of fetal haemoglobin (HbF) does significant interference start? Presence of such variants should always be communicated for appropriate interpretation.
   c. Sample preparation should be looked into, especially for certain kit methods requiring manual preparation of lysate. These have higher chances of human error.

3. Assess instrument for any mechanical or maintenance related problems.
4. Check reagent & buffers for stability, expiry & adequacy of quantity.
5. Review analytical quality indicators: The coefficient of variation of the internal quality controls should be less than 3% and that of external quality assurance should be less than 5%. If in doubt, new vials of controls may be reconstituted and run for confirmation rather than repeating same controls.
6. If a problem lies within the analytical phase, verification of calibration or recalibration may be required.
7. When lab errors are ruled out and repeat testing gives same results, a repeat sample is usually required to further confirm and exclude pre-analytical variables.
8. Repeating the analysis using a different technique, if available, may also rule out method related errors.

1.3 Confirmed Low HbA1c. Now what?

It is safe to say that insulin metabolism, even in non-diabetics, is altered in kidney disease and could affect the available concentration of glucose RBCs are exposed to. Chronic kidney disease ability to causes hypoglycaemia [3] independent of other factors like insulin therapy further extends evidence on the effect of kidney on glucose homeostasis. The two main factors that affect the percentage of haemoglobin that will end up being glycated is the haemoglobin in an RBC and the glucose concentration in the blood [4]. Therefore, any factors that affects either of these may influences the final result.
1.3.1 Well known factors

1. First and second trimester of pregnancy due to high erythropoiesis and RBC turnover.
2. Blood transfusion.
3. Decrease in the lifespan of the RBC will decrease the duration of exposure to glucose for glycation in spherocytosis, example: haemolytic anemia, blood loss etc.
4. Haemoglobin variants depending on the method being used may give low or high values.

Dealing with these conditions, it would be reasonable to further investigate the glycemic control in such patients using other tests like glycated albumin for diabetes [5]. Investigating the cause of low HbA1c may also warrant additional investigation like complete blood count with peripheral blood smear for anemia, bilirubin levels for any signs of hemolysis that affect erythrocyte lifespan and haemoglobin electrophoresis to diagnose any undetected hemoglobinopathies that may need treatment.

1.3.2 Less known factors

A confirmed value of low HbA1c should trigger multiple possibilities.

1.3.2.1 Anaemia

- Factors like spherocytosis or haemolytic anemia are well established causes that lead to a low haemoglobin due to RBC destruction or reduced erythrocyte lifespan [6].
- Macrocytic anemia: It has been observed that anemia with a high mean corpuscular volume leads to a decrease in the concentration of HbA1c. The exact mechanism of why this occurs is not completely understood, but is strikingly opposite to the high HbA1c values seen in mircocytic iron deficiency anemia [7].
- Therapy: Iron therapy used for the treatment of iron deficiency anemia has been seen to be accompanied by a decrease in HbA1c levels.

2. LIVER DISEASE

Anemia is most likely caused by hypersplenism or hemolysis associated with end-stage liver disease, or persistent GI blood loss from portal hypertensive gastropathy. Since the cause of anaemia in patients with decompensated cirrhosis is complex, our retrospective study was unable to examine the impact of different kinds of anaemia on HbA1c levels in these individuals.

Cirrhosis and severe liver disease have been linked to an elevated risk for hyperglycemia and diabetes mellitus. There is a substantial difference in the diagnostic yield of standard tests used to diagnose diabetes and insulin resistance in patients with liver disease and that of the general population. However, HbA1c in people with cirrhosis is neither accurate nor dependable.

Whether it be due to alcohol or hepatitis, a compromised performance of the liver affects both blood glucose and the haematological picture of a patient. Following are the various factors at play that bring about a decrease in the glycation of haemoglobin [8]:

- **Altered Glucose Homeostasis**: Liver is the main organ responsible for regulating blood glucose levels, it is involved in gluconeogenesis, glycogenolysis and has an important role in insulin metabolism. An impaired liver will lead to compromised glucose homeostasis, one of the major determinants of glycated hemoglobin.

- **Low Insulin clearance**: Liver is responsible for removal of 50% of the insulin entering through the portal circulation. Reduced hepatic function and formation of portosystemic shunts hinders this process leading to decreased hepatic clearance. Although many other factors affect hepatic clearance of insulin, this may be one of the contributing factors to an altered blood glucose balance in liver disease.

- **Bleeding**: Blood loss leads to a fall in HbA1c due to the reduced lifespan of erythrocytes and a poor coagulation profile leads to a higher probability of bleeding, which is further accentuated by the presence of portal hypertension & varices.

- **Hypersplenism**: Apart from bleeding, portal hypertension may lead to hypersplenism which adds to number of red blood cells being destroyed.

- **Macrocytic anemia**: is a common finding in liver disease which has been found to be damage and as previously discussed, a high MCV leads to a lower HbA1c.

- **Therapy**: Patients suffering from hepatitis C usually have treatment related contributions to a lowered glycated haemoglobin value via the toxic effects of Ribavarin [9]. Toxic
accumulation of the phosphorylated drug in the RBC depletes the cells of available adenosine triphosphate thereby diminishing the action of the Na-K ATPase pump, causing hemolysis. Interferon is another commonly prescribed drug for hepatitis C that causes anemia by affecting haematopoiesis. Interferon also causes a drop in glucose levels as a consequence of anorexia and weight loss, thereby reducing the glucose concentration available for glycation.

3. KIDNEY DISEASE

Nephropathy is a dreaded complication of diabetes that has a positive correlation with HbA1c. Poor glycemic control would mean a high value of HbA1c to be associated with proportionate renal damage. To add to this a decrease in erythropoietin causes an increase in HbA1c, by increasing lifespan of an RBC. Here it is important to note that the correlation observed between renal insufficiency and high HbA1c is usually a result of studies on diabetic patients with deteriorating renal function. With continued renal damage HbA1c no longer remains a reliable marker for glycemic control as it under reports the glycaemic control in kidney disease [10].

Long-term glycemic stability in diabetes mellitus protects against vascular problems. A1C, a measure of mean blood glucose levels, has been designated as a treatment priority by a large body of data and guidelines.

Chronic hyperglycemia has been implicated in the development of diabetic complications. Many research have been conducted in recent years to determine if glucose variability is a predictor of problems. Within-day and between-day variations in glucose could be established as well as changes in A1C over the course of a year, which could be used to quantify long-term variability. This positive correlation no longer is as strong, warranting the use of alternate methods to assess glycemic control. The mechanisms at play behind this apparent decrease in HbA1c with progressive renal damage are:

- Altered Glucose homeostasis: Apart from liver, kidney is the only other organ that can carry out gluconeogenesis, which is reduced in kidney disease. Acidosis has also been implicated in deterring hepatic release of glucose to maintain homeostasis.

- Insulin metabolism: Kidneys are responsible for the removal of 50% of the systemic circulating insulin and proinsulin. Uremia further reduces the hepatic and muscle clearance of insulin, thereby prolonging the half-life of insulin and its effects [11]. The exact molecular mechanism for this change in insulin metabolism is not well documented, but the uremic toxins of a malfunctioning kidney are proposed to inhibit processes responsible for insulin degradation. This reduces the concentration of available glucose for glycation. At this point it would be incomplete to mention that although insulin clearance is reduced, insulin resistance is also a common finding in kidney disease. Which comes first, though, renal damage or insulin resistance is not exactly clear.

- Decreased RBC Lifespan: Uremia in renal disease leads to a toxic environment for red blood cells, decreasing their survival. It has been seen that uremic serum inhibits the maturation of marrow normoblasts, and increases peroxidative hemolysis [12].

- Therapy: An important component of therapy in patients with renal failure is erythropoietin and iron for anemia. Both these therapies lead to increased erythropoiesis and increase in the number of nascent red blood cells that have very little exposure to glucose for glycation. The net result is an apparent decrease in HbA1c levels.

- Even in diabetic patients with poor renal function, HbA1c values decrease by 1.5% in chronic renal disease and end stage renal disease making it a less reliable marker for diabetes [13]. If looked with a different perspective after a certain point HbA1c is no longer being influenced by glucose levels in diabetes and the lack of correlation of HbA1c values with other indices of glycemic control could be a marker of deteriorating renal function.

4. DRUGS

All hemolytic drugs have the ability to reduce the HbA1c by shortening the lifetime of erythrocytes, resulting in an increased proportion of younger cells in the blood stream. Drugs like dapsone and ribavirin have been seen to lower HbA1c in this method. Various drugs should be considered
while interpreting HbA1c, especially when levels are lower than expectation.

- To recapitulate, Ribavarin, Interferon used for the treatment of hepatitis C, Iron and Erythropoietin used for the treatment of anemia alter HbA1c levels by increasing the number of young cells.
- Dapsone [14]: Dapsone reduces HbA1c by reducing the lifespan of RBCs and this effect is proportional to the dosage of the drug. Dapsone also causes the oxidation and haemoglobin to methemoglobin which may cause interference if HPLC is used as the method of estimation.
- Aspirin, Vitamin C & E have also been implicated in inhibiting the glycation of HbA1c, but such effects from vitamins are usually observed above the prescribed pharmacological doses. Aspirin in low doses inhibits glycation but chronic use of larger doses leads to acetylation of haemoglobin which on estimation by HPLC leads to a significant yet clinically irrelevant false positive values.
- Anti-retroviral agents: Patients undergoing treatment for HIV show falsely low values of HbA1c. These do not correlate with other methods of glycemic control, such as fasting blood glucose levels. As a result of toxicity from nucleoside analogues, sub clinical hemolysis is implicated as the cause of low erythrocyte survival that increase in the population of young erythrocytes, not exposed to glucose for the same amount of time. Macrocytic anemia is also a common finding in patients on treatment with zidovudine, stavudine, and lamivudine. Although macrocytosis was not directly held responsible for contributing to a low HbA1c level in these studies, as previously mentioned macrocytosis is found to be associated with low HbA1c levels.

In the National Health and Nutrition Examination Survey III low glycated haemoglobin emerged as a cause of concern in the healthy population leading to increased mortality. Other studies having similar findings attributed the reason to be autoimmune activation [15]. Further meta-analysis revealed that the apparent increase in all-cause mortality could be attributed to confounding factors like anemia, liver disease, race and ethnicity or to the inclusion of prediabetic individuals in the study of a healthy population. If HbA1c is considered in totality including all the possibilities that are capable of influencing it, a decreased value will open further conditions and diagnosis to explore, especially if it can be a potential indicator of an undiagnosed pathology. Instead of labelling other factors affecting HbA1c as confounder they can be labelled as contributing factors and modify the way HbA1c is being utilized as a marker. With such a possibility, the association of all-cause mortality with low HbA1c would allow a broader exploration in the various possibilities and their treatment for the benefit of the patient. A low value of HbA1c for example has been attributed to general poor health, inflammation, and malnutrition and weight loss. Similarly the correlation that HbA1c has with red blood cell indices also makes it a good marker to assess the degree of haemolysis. The role of HbA1c has been restricted to diabetes and glycemic control. More studies are required to establish the role of this marker in the vast expanse of diseases in which it is affected and therefore can be utilized.

5. CONCLUSION

Ubiquitous use of HbA1c has allowed the surfacing of values towards the lower end of the spectrum. Apart from being laboratory errors and confounders, certain factors should be considered to review HbA1c as a diagnostic marker. Being an easily available test, HbA1c has much more to offer than just a marker of glycemic control. Whether its role expands in future medicine, considering all the factors discussed, it is quintessential that awareness regarding low values or lower than expected values are interpreted with much needed caution. At this point it would be incomplete to mention that although insulin clearance is reduced, insulin resistance is also a common finding in kidney disease. If HbA1c is considered in totality including all the possibilities that are capable of influencing it, a decreased value will open further conditions and diagnosis to explore, especially if it can be a potential indicator of an undiagnosed pathology. To add to this a decrease in erythropoietin causes an increase in HbA1c, by increasing lifespan of an RBC.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

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