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Bursting the bubble: hereditary spherocytosis masking poor glycemic control

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Abstract: Hemoglobin A1c (HbA1c) is a very common measure utilized to diagnose diabetes and to monitor the level of glycemic control during the course of management. Despite the high utility of HbA1c, it has some limitations. Physiological conditions that affect the lifespan of red blood cells (RBCs) can falsely elevate or lower HbA1c results. In this case report, we present a case of a patient who was found to have hereditary spherocytosis (HS) after developing nephrotic range proteinuria. The patient had diabetes that was previously thought to be well controlled, but his HS was masking his poor glycemic control. This case highlights the importance of understanding the limitations of the HbA1c in managing patients with diabetes.

Keywords: benign hematology; diabetes; hereditary spherocytosis.

Hemoglobin A1c (HbA1c) testing has become a standard for diabetes management, yet it does have pitfalls. HbA1c can be falsely elevated or lowered by multiple factors, including hemolytic disorders, hemoglobin variants, pregnancy, and blood transfusions [1–4]. It is important to keep these factors in mind when treating patients with diabetes mellitus to avoid over- or undercorrection of glucose in response to inaccurate HbA1c values. To attract attention to the limitations of HbA1c testing, we present a case of falsely low HbA1c values due to hereditary spherocytosis (HS).

Case description

A 65-year-old man with a history of well-controlled hypertension and type 2 diabetes mellitus (diagnosed at age 40) was incidentally found to have renal dysfunction and proteinuria in November 2016. This was discovered during routine lab work including a basic metabolic panel and a urinalysis with microalbumin/creatinine ratio. His serum creatinine was 1.8 mg/dL (baseline, 0.9 mg/dL) and 24 h urine protein of 3.3 g. His vitals were within the normal limits with blood pressure consistently near 130/70 mmHG and body mass index (BMI) of 32 kg/m². The physical exam was unremarkable without edema. The patient was also noted to have long-standing anemia, for which he was seeing a hematologist. An extensive evaluation for his anemia was unremarkable and included direct and indirect Coombs tests for autoimmune hemolytic anemia as well as upper and lower endoscopic evaluations.

Further evaluation for his proteinuria, including a hepatitis panel, HIV and autoimmune studies, and C3/C4 levels, were within normal limits. His medication regimen consisted of metformin 850 mg twice daily, insulin glargine 60 units once daily, and lisinopril 40 mg daily. Although he did have some documented random blood glucoses in the mid-2000s, he was not checking his home blood glucoses regularly, and his HbA1c of 5.3% was indicative of well-controlled diabetes mellitus. His blood pressure was also well-controlled at the time, so the hypertensive and diabetic nephropathy were low on the differential diagnosis, and renal biopsy was being considered.

A blood smear for his anemia of unknown etiology revealed spherocytes. At this time, more family history was elucidated and revealed that his sister had previously undergone a splenectomy for an unknown blood condition. This raised suspicion for the diagnosis of HS and prompted further evaluation through an osmotic fragility study, which was borderline but not diagnostic. A further red blood cell (RBC) Band 3 study by flow cytometry was abnormal, confirming the diagnosis of HS. The patient was referred and underwent a splenectomy for definitive treatment. The patient's HbA1c values after splenectomy were 8.5–9.5%, with fasting glucoses near 200 mg/dL. His

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insulin regimen was intensified to insulin glargine 72 units daily and insulin aspart 15–20 units three times per day with meals, based on his pre-meal glucose level. Over the following 12 months, his HbA1c improved to <7.0%, with fasting blood glucoses near 100 mg/dL. Our patient experienced a significant improvement in his degree of proteinuria after intensifying his insulin regimen. His 24 h proteinuria improved to 155 mg, and his serum creatinine has since remained stable near 1.5 mg/dL.

Prior literature has demonstrated that very strict glycemic control can lead to a 25–30% decrease in proteinuria, although this degree of proteinuria reduction was not expected. There was no change in his antihypertensive regimen, including his angiotensin converting enzyme (ACE) inhibitor. Although insulin has also been shown to improve glomerular hyperfiltration, which leads to reduced proteinuria in animal models, there is a paucity of literature assessing this phenomenon in human models. Further trials are needed to evaluate the effect of both insulin use and reduction of HbA1c regarding the effect on proteinuria reversal [5]. Because at least part of his renal disease was now thought to be related to diabetic nephropathy and his degree of proteinuria had improved, kidney biopsy was not pursued.

Discussion

There are several ways to screen and diagnose someone with diabetes mellitus. In a patient with symptoms of hyperglycemia, a diagnosis can be made with a random plasma glucose ≥ 200 mg/dL. In patients without symptoms of hyperglycemia, two abnormal test results from a single testing method or two individual testing methods are required. These results include a fasting plasma glucose ≥ 126 mg/dL, plasma glucose ≥ 200 mg/dL 2 h after a 75 g oral glucose load, or HbA1c $\geq 6.5\%$ [1]. HbA1c has remained a highly-utilized biomarker for glycemic control since its inception in the 1970s, and its role broadened in 2010 after the ADA added HbA1c criteria for both diagnosis and management goals. HbA1c is a measurement of the amount of glycated hemoglobin found within RBCs over the course of an RBC's lifespan (approximately 120 days). It therefore provides an indirect measurement of estimated average glucose over a 3 month period. Values 5.7–6.4% indicate prediabetes, and values $\geq 6.5\%$ indicate diabetes mellitus [1]. Utilizing HbA1c as a method to diagnose and monitor diabetes has several advantages: no fasting is required, point of care testing is available, and there is less day-to-day variability from factors including stress, diet, and illness [5]. However, as seen in

Table 1: Common causes of falsely lowered and falsely elevated HbA1c.

Falsely lowered HbA1c	Falsely elevated HbA1c
– Acute blood loss	– Iron-, B12-, or folate-deficiency anemia
– Hemolytic anemia	– Asplenia
– Splenomegaly	– Uremia
– Hemoglobinopathies (e.g., sickle cell)	– Hyperbilirubinemia
	– Hypertriglyceridemia

the case above, there are factors independent of serum glucose levels that can influence HbA1c values.

HbA1c is a measurement that depends on the glycation of RBCs that occurs continuously over a RBC's lifespan and is proportional to the ambient glucose concentration [2, 3]. Therefore, conditions that affect RBC turnover and lifespan can alter HbA1c values [2, 4]. For example, acute or chronic blood loss, hemolytic anemia, erythropoietin (EPO) administration, and splenomegaly can all cause falsely lowered HbA1c results due to RBC destruction, decreased RBC lifespan, and/or increased turnover as seen in the patient described above [2, 6]. Pregnancy can lower HbA1c by reducing RBC lifespan and increasing new RBC production due to elevated EPO levels, and recent blood transfusions lower HbA1c by introducing new RBCs that have not yet been bound by glucose [2, 6]. Conversely, iron-, B12-, and folate-deficiency anemias, as well as aplastic anemia and asplenia, can lead to falsely elevated HbA1c values by prolonging RBC lifespan via a decrease in turnover and/or production [2, 6]. Just as elevated glucose levels result in glycation of hemoglobin, products of urea can also bind to hemoglobin to form carbamylated hemoglobin. Because the binding of these two reactions involves the same amino groups and produces a similar charge, carbamylated hemoglobin can be difficult to differentiate from glycated hemoglobin and contributes to a falsely elevated HbA1c on current testing methods [1].

Finally, HbA1c results may be inaccurate in hemoglobin variants such as sickle cell disease, thalassemia, Hemoglobin C, and Hemoglobin E [2, 4, 6]. These hemoglobin variants have the potential to alter HbA1c in three main ways: influencing the degree of glycation, increasing the risk of hemolysis, and affecting chromatography peak measurements (hemoglobin variants can create an abnormal peak on chromatography, making HbA1c estimation unreliable) [2, 4]. The National Glycohemoglobin Standardization Program (NGSP) has developed different assays meant for different hemoglobin variants [7]. However, even if the assay does not interfere with a specific variant, it is possible that some other underlying anemia or

Table 2: Alternative testing methods to HbA1c that can help determine the level of glycemic control.

Test	Details	Limitations
Glycated fructosamine	<ul style="list-style-type: none"> Measures all glycated serum proteins Not affected by RBC lifespan Reflects glucose levels over past 2–3 weeks 	<ul style="list-style-type: none"> Affected by serum albumin concentration Albumin-losing conditions (such as advanced diabetic nephropathy) can result in falsely lowered values
Glycated albumin	<ul style="list-style-type: none"> Measures proportion of glycated albumin to total albumin Not affected by albumin levels Not affected by RBC lifespan Reflects glucose levels over past 2–3 weeks 	<ul style="list-style-type: none"> Conditions causing decreased albumin metabolism (e.g., cirrhosis, hypothyroidism) can cause falsely elevated values Elevated BMI associated with lower values compared to HbA1c, but it is not known why
1,5-anhydroglucitol (1,5-AG)	<ul style="list-style-type: none"> 1,5-AG normally competes with glucose for proximal tubule reabsorption Within 24 h of elevated glucose levels, 1,5-AG levels start to rise Reflects glucose over past 1–2 weeks 	<ul style="list-style-type: none"> Values affected by alterations in renal function
Continuous glucose monitoring	<ul style="list-style-type: none"> Can accurately monitor glycemic variability within and between days 	<ul style="list-style-type: none"> Relatively expensive and not easily accessible Requires significant patient cooperation

BMI, body mass index; RBC, red blood cell.

a condition altering RBC turnover or hemolysis can result in inaccurate HbA1c values. It is also important to note that the presence of an abnormal hemoglobin gene may have the potential to alter HbA1c even without overt hemoglobinopathy. A study conducted in 2017 demonstrated that the presence of the sickle cell trait alone in African Americans was associated with significantly lower HbA1c values when compared to those without the trait [8]. Table 1 lists several causes of falsely elevated and falsely lowered HbA1c levels [2–4, 6].

The osteopathic philosophy embraces the principle that a person is a combination of mind, body, and spirit. Just as it is essential to treat a patient as a “whole” person instead of a singular disease state, it is essential for clinicians to look at a patient’s entire clinical picture when interpreting HbA1c and to assess which factors may be contributing to alterations in true values. Whenever HbA1c values do not correlate with plasma glucose levels, it is important to consider whether some underlying factor is contributing to the abnormal values and whether a different method for monitoring glucose should be utilized.

Alternatives for monitoring glucose control include fasting blood glucose, random blood glucose, daily glucose logs or glucometer readings, and continuous glucose monitoring (CGM). Fasting and random blood glucose levels can be beneficial for the screening and diagnosis of diabetes; however, these are spot values that may not accurately portray the overall glycemic control. Daily glucose logs and glucometer readings are beneficial in that they can provide multiple values for a single day. Continuous glucose monitors are also able to provide multiple readings in a single day

and can transmit blood glucose data to a computer or phone to help visualize glucose trends. Some CGM devices are also able to alert a person if their blood glucose is too low or too high without the person needing to check. Continuous glucose monitors and glucometer readings provide more quantitative blood glucose data than HbA1c, which can allow for more frequent adjustment of treatment regimens based on an individual’s needs. However, this increased data collection requires more effort from the patient, and values can vary significantly on a day-to-day basis depending on diet, activity, stress, illness, and timing of glucose readings in relation to a patient’s previous meal.

Other alternative testing methods to HbA1c have also been developed. For example, fructosamine is a measurement of glycated serum proteins, often albumin. Because albumin has a half-life of 2–3 weeks, fructosamine values reflect the estimated average glucose over 2–3 weeks. Fructosamine is not affected by RBC lifespan and allows for faster measurements of glucose control after adjusting for treatment regimens, yet it has its own limitations [2, 6, 9, 10]. Fructosamine will be falsely low in patients with low albumin, such as in those with liver disease, protein losing enteropathy, or nephrotic syndrome, as well as in conditions with increased protein metabolism such as hyperthyroidism, hyperuricemia, and hypertriglyceridemia. Conversely, conditions with decreased protein metabolism (hypothyroidism) can cause falsely elevated HbA1c values [2, 6]. At this time, the American Diabetes Association does not include fructosamine measurements as a way to screen for diabetes [1]. Instead, it is often utilized in patients with conditions that limit the utility of HbA1c. Table 2 lists several

other alternatives to HbA1c testing that may provide a better overall picture of a patient's level of glycemic control, along with the major limitations of each test [2, 6].

Conclusions

HbA1c testing remains a commonly utilized method for diagnosing and directing diabetes management given its ease of testing, widespread availability, and low cost. However, it is important that clinicians keep in mind the spectrum of scenarios that may alter HbA1c values, because this may result in over- or undertreatment of diabetes, resulting in poor glycemic control, as was the case with the patient presented in this case report.

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