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CASE REPORT

Glycosylated haemoglobin: a false sense of security

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SUMMARY

We report the unusual case of a patient found to have a low glycosylated haemoglobin (HbA1c) despite having recently been diagnosed with diabetes mellitus type 2. The patient, who was not anaemic, with no symptoms or family history of haematological conditions, was subsequently found to have an elevated reticulocyte count, inferring increased red cell turnover as the culprit for the discordant HbA1c result. A diagnosis of hereditary spherocytosis was made based on characteristic peripheral blood film appearances and confirmed by eosin-5-maleimide binding test. Exposure of an undiagnosed haemolytic anaemia by virtue of a low HbA1c is uncommon. However, conditions that distort HbA1c measurements are not infrequent. This case should serve to remind clinicians of the limitations of HbA1c in specified situations, and to remain vigilant when interpreting results.

BACKGROUND

Since 2011, glycosylated haemoglobin (HbA1c) has been endorsed by WHO for the screening, diagnosis and monitoring of diabetes mellitus.[1] HbA1c measurement provides a reflection of a patient's glycaemic control over the preceding 8–12 weeks and correlates with the development of diabetic complications.[2] Despite this, its limitations are well documented in patients with certain haematological and genetic conditions.[3 4] In these patients, HbaA1c may give false reassurance, and it is advisable that, when such conditions are recognised, alternative methods are used to diagnose and monitor diabetes.[5 6]

This report highlights the limitations of using HbA1c as a diagnostic tool in patients with increased red blood cell turnover. In this case, an unexpectedly low HbA1c unmasked a compensated haemolytic anaemia due to previously undiagnosed hereditary spherocytosis (HS). While HS in this patient has been categorised as mild and is unlikely to require any intervention, the haemolysis is of clinical significance since it inadvertently delayed the diagnosis of diabetes.

CASE PRESENTATION

A 37-year-old Caucasian woman was referred to haematology outpatients for investigation of a reticulocytosis.

Several months prior to presentation, she had been diagnosed with diabetes mellitus type 2 based on high blood glucose measurements. Her HbA1c, however, which had been measured on several occasions, was unexpectedly low. Further bloods revealed a normal haemoglobin (Hb) level with a high reticulocyte count, a raised mean corpuscular haemoglobin concentration (MCHC) and a slightly elevated bilirubin. Her folate level had been low in the past.

She reported no symptoms of anaemia or jaundice. She had no bleeding.

She has hypothyroidism, for which she takes thyroxine and type 2 diabetes mellitus, for which she takes metformin. Her other medications include atorvastatin and lansoprazole.

She does not smoke or drink alcohol.

She reported no family history of haematological conditions, however, her mother did have anaemia in later life and various comorbidities. She has a sister and four children, all who are well, with no symptoms of anaemia or jaundice.

Her physical examination revealed no palpable splenomegaly, lymphadenopathy or detectable jaundice.

A haemolysis screen was arranged.

INVESTIGATIONS

At the time of referral, blood tests showed Hb 12.2 g/dL (11.5–16.0 g/dL), haematocrit 0.354 (0.370–0.470), mean cell volume 86.6 fL (78.0–100.0 fL), red cell distribution width (RDW) 17.2% (11%–16%), reticulocytes 488×10 (10–100×10) with a reticulocyte percentage of 11.9%. MCHC had previously been elevated, but at time of referral was 345 g/L (310–350 g/L). Urea and electrolytes were unremarkable. Liver enzymes were normal, but total bilirubin was slightly elevated at 22 μmol/L (0–21 μmol/L). Folate had been low in the past, but at the time of referral measured 3.6 μg/L (3.1–20.5 μg/L).

The haemolysis screen was positive: haptoglobin <0.08 g/L (0.40–1.60 g/L) and lactate dehydrogenase (LDH) 277 U/L (125–243 U/L). Direct Coombs test was negative.

The peripheral blood film showed numerous spherocytes and marked polychromasia.

Elevated morning glucose results had been recorded on a number of occasions, for over 2 years prior to diagnosis of diabetes: 6.3–8.3 mmol/L (reference range 3.3–6.0 mmol/L).

HbA1c had been measured multiple times in the preceding 3 years. It had been as low as 11 mmol/mol in 2015, and was 19 mmol/mol (20–41 mmol/mol) at the time of referral.

Abdominal ultrasound revealed splenomegaly of 14.5 cm.

A blood sample was sent to Sheffield Children's Hospital in a potassium ethylenediaminetetraacetic acid (EDTA) container, and subjected to the

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eosin-5-maleimide binding test. A fluorescence ratio of 0.69 was recorded (ratio consistent with a diagnosis of HS <0.85).

TREATMENT

In most patients with asymptomatic HS, treatment is not required as the haemolysis is compensated. However, these patients are vulnerable to some infections, for example, parvovirus B19, which can cause an aplastic anaemia, resulting in a need for blood transfusion support.

Patients with symptomatic anaemia due to uncompensated haemolysis may need blood transfusions to maintain Hb levels. Those requiring regular transfusions are considered for splenectomy. Haemolysis can also predispose to gallstones and cholecystectomy may be indicated. Patients with HS often need oral folic acid supplementation to meet increased requirements.

OUTCOME AND FOLLOW-UP

Once a diagnosis of HS was confirmed, the patient was contacted to arrange screening of her children and sibling.

DISCUSSION

In health, the lifespan of a mature red blood cell is approximately 120 days.[7] The longer an erythrocyte remains in the circulation, the greater the exposure of adult HbA to glycosylation.[2] Conditions that reduce the lifespan of red blood cells, such as haemolysis, thalassaemia, sickle cell anaemia and acute or chronic blood loss, result in lower HbA1c measurements. The opposite occurs when erythrocytes have a prolonged lifespan, as seen postsplenectomy and in iron or vitamin B_{12} deficiency.[8]

HS is an inherited disorder of red blood cells resulting in membrane instability and loss of surface area. The spherical shape and reduced deformability renders cells susceptible to damage and splenic sequestration, producing a haemolytic anaemia.[9]

HS can be seen in all ethnic groups; the frequency commonly reported to be between 1 in 2000 and 1 in 5000 persons, with a predominance in Northern Europeans.[10] Many genetic defects have been identified, with families often displaying unique mutations.[11] While the majority are found to be autosomal dominant (75%), the inheritance pattern varies depending on the molecular cause, and recessively inherited and de novo mutations also occur (25%).[10 12] In some cases, the genetic defect is undetermined.[13 14] The membrane changes in HS result from mutations in genes encoding proteins integral in maintaining the vertical links between the inner cytoskeleton and the outer lipid bilayer. The most common defects are found in ankyrin (ANK1), spectrin (SPTA1, SPTB), Band 3 (SLC4A1) and less commonly Band 4.2 (EPB42) proteins, with studies varying in the reported prevalence of these mutations.[15]

The clinical phenotype varies from a compensated or mild haemolytic anaemia to a severe neonatal haemolysis. Patients may experience symptoms of anaemia, jaundice, splenomegaly and gallstones. One study of 300 patients found that there was no clear clinical correlation between genetic defect and disease severity, although it was noted that spectrin/ankyrin deficiency was more often diagnosed in childhood and more frequently resulted in splenectomy.[15]

The blood picture may guide clinicians to a diagnosis of HS. Characteristic spherocytes and the presence of immature red cells are seen on peripheral blood smear. The RDW and MCHC are often increased. The Hb is low in patients where the red cell loss is greater than erythropoiesis. Unconjugated bilirubin may be elevated, although in healthy states, this is usually mild since it is effectively processed and excreted by the liver. Folate levels may be low due to increased requirements.

Establishing a diagnosis of HS is important as various insults can exacerbate the anaemia. Infection with parvovirus B19 temporarily inhibits erythropoiesis and can result in aplastic crisis. Patients with chronic haemolytic anaemia rely greatly on the ability of the bone marrow to compensate by increasing red blood cell production, and even a transient halt in erythropoiesis can be reflected in the blood count.

A similar case is described by Arnold and McGowan.[16] They report a delay in diagnosis of diabetes in a patient with known HS, owing to false reassurance from HbA1c levels. Their case demonstrates that clinicians can be misled even when conditions known to interfere with HbA1c are pre-existing. Encouraging clinicians to think physiologically about the test will increase the chance of identifying potential confounders in the interpretation of the result. This becomes more complex, as seen in our case, when the underlying haematological condition was not only undiagnosed, but also unsuspected, since the haemolytic anaemia was compensated and the patient asymptomatic.

While we found relatively few papers reporting a delay in diagnosis of diabetes owing to an undiagnosed haemolytic anaemia, reviewing the literature did reveal an abundance of papers highlighting the limitations of HbA1c assays in patients with Hb variants.[17–20] Variations in Hb pose an analytical rather than a clinical distortion of HbA1c. Differing assays and techniques performed by laboratories, each with limitations and potential for interference from Hb variants, make prediction of an erroneous HbA1c result challenging.[21] An example is described by Chessler and Lee, who report a near misdiagnosis of diabetes in a patient with an Hb variant.[22] In this case, the discrepancy in HbA1c measurement was attributed to a change in the laboratory instrument used in the assay. Initially, a deceptively low HbA1c measurement was recorded owing to the interaction of the mutated alpha chain with the old laboratory instrument. Once the analyser was updated, the true HbA1c result was revealed, leading to a diagnosis of diabetes.

In patients known to have increased red cell turnover or abnormal Hb, National Institute for Health and Care Excellence advise the use of fructosamine as a means to monitor glycaemic control. This gives an indication of a patient's glycaemic control over the preceding 2–3 weeks.[23]

While it is possible to encourage vigilance among clinicians when conditions known to disrupt HbA1c measurement are present, misleading results will still occur in patients that have undiagnosed confounders.

Learning points

- Understand the limitations and think physiologically about glycosylated haemoglobin (HbA1c) measurement, interpreting each result in context of the clinical situation.
- Unexpectedly low HbA1c may provide false reassurance in patients with acute or chronic blood loss, haemoglobinopathies and haemolytic anaemia.
- It is difficult to interpret HbA1c in patients with iron deficiency anaemia, B_{12} and folate deficiency and postsplenectomy, as the results may be higher than expected.

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