CASE REPORT

Alarming increase in HbA1c and near misdiagnosis of diabetes mellitus resulting from a clinical laboratory instrument upgrade and haemoglobin variant

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SUMMARY

A 29-year-old woman was referred for new-onset diabetes mellitus after her glycosylated haemoglobin (HbA1c) was found to be 10.2%. Three years earlier, the patient's HbA1c—measured by the same clinical laboratory—had been 5.5%. The newer HbA1c was discordant with fasting glucose levels and a lack of diabetes-associated symptoms. The laboratory reported that their assay methodology remained unchanged and also that no haemoglobin variants were detected. Further investigation, however, revealed, first, that the patient carried a haemoglobin alpha chain mutation (Hb Wayne) that can sometimes cause assay interference and, second, that although the laboratory's assay methodology had not changed, their assay instrument had. Depending on assay methodology, haemoglobin variants can cause HbA1c assay interference and the presence of these variants may not be detected by the performing laboratory. Interference may not only be dependent on assay methodology but also on the assay instrument used.

BACKGROUND

This case is a reminder of potential hazards in the interpretation of glycosylated haemoglobin (HbA1C) results and the need to investigate results—even reproducible results—that, at first, seem not to make sense. Knowledge of these hazards should aid in the interpretation of HbA1c results, preventing misdiagnosis of diabetes and unnecessary treatment for the disease. There are over 1000 haemoglobin variants, including many uncommon ones, and—as this case teaches—these can cause significant interference with one of the most common types of HbA1C assay: ion-exchange high-performance liquid chromatography (HPLC).¹ The instruments that carry out this assay can detect haemoglobin variants, but the clinical laboratory in this case reported that none had been detected and therefore the presence of such a variant was not the cause of a surprisingly elevated HbA1c. Another important lesson, then, is that haemoglobin variants can sometimes go undetected by assay instruments, leading to erroneous HbA1c results by clinical laboratories. Clinical laboratories should be on the lookout for clues in HPLC chromatograms, in addition to the presence of abnormal peaks, that haemoglobin variants may be present. Even if reproducible, HbA1c results—and, for that matter, laboratory

results in general—discordant with other findings merit further investigation. Lastly, it is important to understand both that different HbA1c assay methodologies are subject to assay-specific interferences and—as this case illustrates—that the effect of interferences can also be instrument specific.

CASE PRESENTATION

A 29-year-old woman was referred to an endocrinology clinic by her university's student health centre for newly diagnosed diabetes. Because her body mass index (BMI) was 32, a HbA1c test had been ordered during a routine health evaluation. The referral was made after the HbA1c came back at 10.2% and then retested and found to be high again at 10.3%. The patient's mother and maternal uncle both had type 2 diabetes. The patient reported feeling well, with no polyuria, polydipsia, polyphagia or recent fluctuations in weight. The patient denied any history of anaemia or haemoglobinopathy. She did report a history of congenital heart murmur due to benign patent ductus arteriosus. Her only prescription medication was an oral contraceptive. She also used a nutritional supplement containing wheat grass and various vegetables, grains and herbs. Three years earlier, testing ordered by the student health centre had yielded a HbA1c of 5.5%. Physical examination revealed normal vital signs (blood pressure 136/88 mm Hg, heart rate of 76 beats/min) and was unremarkable. There was no acanthosis nigricans, striae, bruises, moon facies, proximal muscle weakness, thyromegaly or vitiligo. In addition to testing the HgA1c twice, the primary care clinic had also tested fasting glucose, which was 87 mg/dL. Complete blood count results were all in the normal range, including a haemoglobin of 14.4 g/dL and mean corpuscular volume of 82.5 fL.

INVESTIGATIONS

The two recent HbA1c levels suggested that diabetes mellitus with severe hyperglycaemia had developed within the 3 years after the normal HbA1c result was obtained. The clinical presentation, however, was discordant, including, notably, a lack of polyuria and polydipsia and normal fasting glucose. Because of this, prior to initiating treatment for diabetes, HbA1c was measured again at the time of the endocrinology clinic visit using a point-of-care instrument and a sample sent off to the clinical laboratory for repeat testing. The

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HbA1c determined by the clinical laboratory was 10.3%, while the value yielded by the point-of-care testing was 4.9%. This latter value was assumed to be correct, but it was important to be certain in order not to miss an important diagnosis. The clinical laboratory was contacted and confirmed that they had carried out the testing 3 years prior that had yielded a result of 5.5%. The laboratory reported that they had not changed the assay during the 3-year interval: ion-exchange HPLC assay was performed using an instrument made by Bio-Rad, and the instrument had detected no abnormal chromatogram peaks indicative of a haemoglobinopathy. The point-of-care instrument in the endocrinology clinic (Siemens DCA Vantage) determined HbA1c using an immunoassay.

Initially, there was no ready explanation for the increase in HbA1c reported by the clinical laboratory. A thorough literature search for foods or nutritional supplements (such as the one the patient was using) that could cause transient, substantial increases in HbA1c without causing hyperglycaemia yielded no results. A sample of the patient's blood was next sent to an outside laboratory (Mayo Medical Laboratories) for testing using boronate affinity chromatography, which is less susceptible to interference by haemoglobin variants.² This latter technique yielded a HbA1c of 5.0%. The discrepancy between this result and that obtained using ion-exchange HPLC prompted testing for the presence of abnormal haemoglobins. Haemoglobin electrophoresis indeed suggested the presence of a haemoglobin variant. Isoelectric focusing with subsequent confirmation by mass spectrometry (both

performed by Mayo Medical Laboratories) revealed that the patient carried a rare haemoglobin (Hb), a variant called Hb Wayne. Hb Wayne results from a frameshift mutation in the HBA2 gene, one of two genes encoding the haemoglobin alpha chain. Two elongated alpha chain variants are formed: one with an asparagine (Asn) residue at position 139 and another with an aspartic acid (Asp) residue resulting from spontaneous deamination of the Asn residue. The Hb variants carrying these chains are referred to as Hb Wayne1 and Hb Wayne2, respectively.^{3,4}

To determine the reason for the increase in the HbA1c as measured by the clinical laboratory compared with 3 years prior, reconfirmation that the methodology had not changed was requested. These further inquiries revealed that shortly after the earlier HbA1c was measured, the laboratory, in order to increase sample throughput, had upgraded from a Bio-Rad D-10 instrument to the faster Bio-Rad Variant II Turbo. The patient's blood was then tested with both instruments simultaneously, yielding a HbA1c result of 5.5% with the older instrument and 10.2% with the newer. Inspection of the chromatograms (figure 1) shows that while the slower D10 was able to resolve the Hb Wayne1 variant, the Variant II Turbo, although also an ion-exchange HPLC instrument, yielded a chromatogram in which the Hb Wayne1 (Asn 139) and Hb Wayne2 (Asp 139) variants co-eluted with HbA1c and fetal hemoglobin (HbF), respectively. This masked the presence of the haemoglobin variant and yielded an erroneously high HbA1c result (figure 1A).

Figure 1 High-performance liquid chromatography chromatograms. Chromatograms and tables listing peaks generated by the Turbo II instrument (A) and the D10 instrument (B). Tables, from top to bottom, give areas and retention times for peaks in order from left-most to right-most. Hb Wayne isoform 2 (W2) co-elutes with fetal haemoglobin (HbF) (F) on both instruments (peak F+W2). This yields apparent HbF peak areas of 7.8% and 6.7%. Typically, HbF would be <2%. The Turbo II instrument (A) does not resolve Hb Wayne1 (W1), resulting in an apparent glycosylated haemoglobin (HbA1c) peak area of 10.2% (A1c+W1). Because there are no abnormal peaks, the elevated HbF is the only clue as to the presence of an unusual Hb variant. The D10 instrument (B) is slower (note that the last peak elutes at 1.43 min vs 1.02 min with the Turbo II) and yields a separate peak for Hb Wayne1 (W1; labelled 'unknown' in the table). As a result, the A1c peak is not spuriously elevated and the HbA1c result is 5.5%.

OUTCOME AND FOLLOW-UP

The patient was informed that she is a carrier (presumably heterozygous) of Hb Wayne and that, aside from interference with certain HbA1c assays, expression of this variant will not cause anaemia or have any other clinical effects.^{3 4} At the time of her first endocrinology clinic visit, she was advised that her lack of symptoms and her glucose levels were inconsistent with the two recent HbA1c values of over 10% and diagnosis of diabetes mellitus; she was informed that the point-of-care result (4.9%) was more likely to be correct. The subsequent investigations showed conclusively that the alarmingly high HbA1c values that led to her referral were attributable to a haemoglobin variant and that she indeed did not have diabetes.

DISCUSSION

Advances in HbA1c assay technology and standardisation allow HbA1c to be used to diagnose diabetes. Although guidelines for making the diagnosis of diabetes mellitus call for HbA1c to be measured at $\geq 6.5\%$ at least twice (in the absence of unequivocal hyperglycaemia) in order to increase the certainty of the diagnosis, such repetition—as illustrated here—will not prevent an erroneous diagnosis if there is a problem inherent to the assay.⁵ Therefore, it is critical that

practitioners be familiar with the limitations of HbA1c assays and potential interferences.

Here, the assay was ion-exchange HPLC, the second-most common methodology used in clinical laboratories to determine HbA1c.⁶ While this assay is most susceptible to interference from haemoglobin variants, the instruments can often detect the presence of such variants as a result of the presence of abnormal chromatogram peaks. In this case, while an older ion-exchange HPLC instrument was able to separate the Hb Wayne1 protein peak from the normal Hb peaks (figure 1B), the faster elution time of the newer instrument resulted in the overlap of Hb Wayne1 with HbA1c, therefore masking the presence of the Hb variant and leading to the reporting of an artefactually elevated HbA1c (figure 1A).

A prior report—in which Hb Wayne caused a spurious increase in HbA1c—further illustrates the importance of awareness by clinicians and laboratory staff of potential, difficult-to-detect interferences. In this prior case, medical therapy for diabetes was initiated—resulting in repeated hypoglycaemic episodes—before HbA1c was found to be normal using a different assay.⁷ HbF typically comprises less than 2% of Hb in adults. Here, the large HbF peak (caused by overlap with Hb Wayne2; figure 1) could have alerted laboratory staff to consider the possibility that an interfering haemoglobin variant was present. A suggested algorithm to identify interference caused by Hb Wayne calls for the presence of this variant to be considered in samples with HbA1c $>9\%$ and HbF $>7\%$.⁷ An awareness by clinicians that they should use a different assay type to retest HbA1c results discrepant with other clinical findings, however, would provide the strongest protection against diagnostic errors caused by HbA1c assay interference.

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Patient's perspective

I would like to express my sincere gratitude to Dr Chessler and his colleagues for their diligent and investigative work in discovering the cause of my contradictory glycosylated haemoglobin (HbA1c) results and identifying my uncommon haemoglobin mutation. I was confused and concerned when confronted with an extremely high HbA1c, yet normal fasting blood glucose. The subsequent oral glucose tolerance test provided no further insight. I breathed a sigh of relief when my HbA1c came back normal by immunoassay and I felt a profound sense of resolve when Dr Chessler discovered the Hb Wayne mutation. While I wish there was more information about this minimally researched mutation, I am very glad to have an answer to this puzzle.

Learning points

- ▶ Haemoglobin variants—of which there are at least 1000 different types—can cause glycosylated haemoglobin (HbA1c) assay interference.
- ▶ Instruments that are resistant to interference from common haemoglobin variants may nevertheless be susceptible to interference from less common variants.
- ▶ The presence of haemoglobin variants will not always be evident in assay results/chromatograms.
- ▶ An elevated HbA1c result, even if repeated, should be questioned and redetermined using a different methodology if discordant with other clinical findings.
- ▶ HbA1c assay interference not only varies with the assay methodology but also with specific instruments: this is a case in which faster is not necessarily better.

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