A case of haemoglobinopathy and elevated HbA1c

Dr Haval Surchi
SpR in Diabetes and Endocrinology

Dr Rustam Rea
Consultant in Diabetes

1Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), Churchill Hospital, Oxford, UK

Correspondence to:
Dr Haval Surchi, SpR in Diabetes and Endocrinology, Churchill Hospital, Old Road, Headington OX3 7LE, UK; email: haval.surchi@ouh.nhs.uk

Received: 13 September 2016
Accepted in revised form: 26 September 2016

Abstract
A 34-year-old woman was referred to the specialist diabetes service for investigation of fainting episodes. She was otherwise fit and well, spending 10 hours a week cycling, running and swimming. Multiple pathology investigations were undertaken prior to her appointment which were all within normal limits except her HbA1c result which was raised at 77mmol/mol (9.2%). She had no symptoms of hyperglycaemia. Following a review in the diabetes clinic, a suspicion of a spurious HbA1c result arose. Her blood sample was reprocessed using a boronate affinity assay (Alere Afinion analyser) and the result came back as 32mmol/mol (5.1%) compared with an HPLC (Bio-Rad D100) method of 83mmol/mol (9.7%). Further analysis of her haemoglobin (Hb) subtypes showed that she had Hb Wayne.

Hb Wayne is a variant haemoglobin first described in 1976 as an elongated α-chain frame shift variant. It does not produce clinical symptoms and exists as two isoforms: Hb Wayne I (Asn 139) and Hb Wayne II (Asp 139). The prevalence of Hb Wayne trait is unknown; 62 cases over a 16-year period have been reported in a predominantly US population. The abnormal Hb Wayne haemoglobin acts like HbA1c (even though it is not attached to glucose), and this gives an overlapping peak when the separated species are detected and provides a falsely elevated HbA1c value. Copyright © 2017 John Wiley & Sons.

Key words
Hb Wayne; haemoglobin variants; HbA1c; falsely elevated HbA1c

Introduction
Specific haemoglobin (Hb) variants may interfere with the accuracy of HbA1c values depending on the detection method used.

We report one example of such interference and discuss the implications for clinical practice.

Case summary
A female triathlete aged 34 was referred to the specialist diabetes service for investigation of fainting episodes. She had experienced several episodes of hypoglycaemia-like attacks that had become more frequent and increasing in severity to the point where she had almost fainted. Her symptoms included feeling clammy, pale and unsteady; and these were improved by eating. Initially, they occurred once a month but had increased recently to once every day. Some of her symptoms were precipitated by exercise but not on a regular pattern. She did not have a family history of any medical disease and specifically had no family history of type 1 or type 2 diabetes nor of monogenic diabetes. She had two previously successful pregnancies. She was otherwise fit and well, spending 10 hours a week cycling, running and swimming. On clinical examination, her blood pressure was 106/75mmHg, BMI was 21.1kg/m², body weight was 65.5kg, and examinations of chest, heart, abdominal and nervous systems were normal.

Prior to her appointment, a number of investigations were requested including: renal function; liver function test; HbA1c; paired C-peptide (832pmol/L) and blood glucose (4.5mmol/L); thyroid function test (TSH 0.69mU/L 0.30–4.20); prolactin (130mU/L 110–560); cortisol 350 (330nmol/L); and full blood count. All blood tests were within normal limits except her HbA1c result which was raised at 77mmol/mol (9.2%). She had no symptoms of hyperglycaemia. Following a review in the diabetes clinic, a suspicion of a spurious HbA1c result arose.

After discussions with the pathology laboratory her blood sample was reprocessed using initial high-performance liquid chromatography (HPLC; Bio-Rad D100) and also using a boronate affinity assay (Alere Afinion analyser). The HbA1c result
using the boronate affinity assay (Alere Afinion analyser) came back as 32mmol/mol (5.1%) compared with the HPLC (Bio-Rad D100) method of 83mmol/mol (9.7%).

Further analysis of her Hb subtypes showed that she had Hb Wayne. Metformin was discontinued.

The patient is currently under endocrinology follow up. She had short synacthen test which was normal: baseline cortisol 190 and 30-minute cortisol 503nmol/L (normal response is minimal 430nmol/L), and urinary metanephrines were also normal (normetanephrine 454pmol/L [120–1180], metanephrine 216pmol/L [80–510], 3-methoxytyramine <180pmol/L [0–180]). The patient is better now and the attacks are much less frequent.

We have not found any pathological cause for her symptoms and it has been attributed to the low BMI and extreme exercise.

Discussion
HbA1c is widely used for the diagnosis and monitoring of patients with diabetes. Many factors can interfere with the HbA1c value, with multiple mechanisms. A falsely high HbA1c can be caused by reduced erythropoiesis (e.g. iron or vitamin B12 deficiency). The other mechanism is by a direct effect on glycation, e.g. alcoholism, chronic renal failure and decreased intra-erythrocyte pH. Hb variant or chronic exercise.

Key points
- Many factors can interfere with the HbA1c value, with multiple mechanisms.
- The interference can be easily detected by using an alternative analytic method.
- It is important to interpret the results of HbA1c in a clinical context.
- If the result of HbA1c is not in keeping with the presentation, consider assay interference as a cause of the discrepancy.

Table 1. Other haemoglobin variants: their analytic methods and effects on HbA1c results

<table>
<thead>
<tr>
<th>Haemoglobin type</th>
<th>Methods</th>
<th>Effect on HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobins C</td>
<td>Immunoassay (majority of methods)</td>
<td>Falsely elevated</td>
</tr>
<tr>
<td>Haemoglobins AE</td>
<td>Ion-exchange HPLC (majority of methods)</td>
<td>Falsely low HbA1c</td>
</tr>
<tr>
<td>Haemoglobins D</td>
<td>Boronate affinity (majority of methods)</td>
<td>Falsely low HbA1c</td>
</tr>
<tr>
<td>Haemoglobins F</td>
<td>All methods</td>
<td>No specific method data available</td>
</tr>
<tr>
<td>Hb Weesp and Hb Haelen</td>
<td>Ion-exchange HPLC</td>
<td>Falsely low HbA1c</td>
</tr>
<tr>
<td>Hb Le Lamentin</td>
<td>Ion-exchange HPLC</td>
<td>Falsely low HbA1c</td>
</tr>
<tr>
<td>Hb Phnom Penh</td>
<td>Ion-exchange HPLC</td>
<td>Falsely elevated</td>
</tr>
</tbody>
</table>

Conclusion
Haemoglobin variants can interfere with a number of glycohaemoglobin assays. The interference can be easily detected by using an alternative analytic method. It is therefore important to interpret the results of HbA1c in a clinical context and, where the result is not in keeping with the presentation, to consider assay interference as a cause of the discrepancy.

Haemoglobins AE (and some other haemoglobinopathies) contains an amino acid substitution (three amino acids are replaced by eight amino acids), and this changes the charge of the haemoglobin molecule so that it ‘mimics’ the HbA1c species when it is separated by chromatography. The abnormal Hb Wayne haemoglobin acts like HbA1c (even though it is not attached to glucose) and this gives an overlapping peak when the separated species are detected and gives a falsely elevated HbA1c value. In boronate affinity methods, m-aminophenylboronic acid reacts specifically with glucose bound to Hb. This is unaffected by the amino acid substitution in Hb Wayne and so this method tends to demonstrate the least interference from the presence of Hb variants. The prevalence of Hb Wayne trait is unknown; Szuberski et al. identified 62 cases over a 16-year period in a predominantly US population.

A number of other variants of Hb are described resulting in interference with HbA1c analysis. They can cause both erroneously high or low levels of reported HbA1c. See Table 1.

Acknowledgements
Our thanks go to: Elinor Harris, Knowledge Centre Manager and Outreach Librarian, Bodleian Health Care Libraries, University of Oxford; and the consultant team in the Department of Clinical Biochemistry, John Radcliffe Hospital – Dr Nishan Guha, Dr Brian Shine, Dr Reza Morovat, Dr Tim James.

Declaration of interests
There are no conflicts of interest declared.

References