A 52 year old male patient was found to have an HbA1c of 26 mmol/mol (4.5% NGSP) on the primary method of analysis. Our laboratory uses a High Pressure Liquid Chromatography (HPLC) analyser (Bio Rad Variant), and an abnormal chromatogram was noted suggesting a possible analytical artefact, or a potential haemoglobin variant. HbA1c measured on the same sample using an alternative point-of-care instrument (DCA2000), that uses immunoassay, was measured to be 50 mmol/mol (6.7% NGSP) (Table 1) (1).

This raises key questions regarding the nature of HbA1c measurements:
1. Which HbA1c (if either) is the correct result?
2. What is a haemoglobin variant and how can we investigate this further?
3. If HbA1c was being used for diagnosis what implications does this have?

Table 1. Glucose and HbA1c results by different methods in a patient with HbD (ref. 1).

<table>
<thead>
<tr>
<th>Glucose (random) (mmol/L)</th>
<th>HbA1c by HPLC [Bio Rad Variant] (mmol/mol)</th>
<th>HbA1c by Immunoassay [DCA 2000] (mmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.0</td>
<td>26</td>
<td>50</td>
</tr>
</tbody>
</table>

Reference range HbA1c = 20-40 mmol/mol.

DISCUSSION

Clearly, there is discordance in results between the two analytical methods and either, or both, could be misleading. As an abnormal chromatogram was detected, it suggests there may be problems with the measurement on the primary analyser (Bio Rad Variant). To determine the true result, HbA1c could be measured using alternative analytical methods; including boronate affinity chromatography, capillary electrophoresis (2), or mass spectrometry (3). However, the first question is which HbA1c most closely matches with the clinical picture? In this patient, the plasma glucose was 15.0 mmol/L, suggesting that the higher HbA1c result (from the point-of-care instrument) was more likely to be the true result. Notwithstanding, the laboratory must find out more about the haemoglobin variant with the involvement of clinicians before we may be certain this is the case.

In 2010, 950 haemoglobin variants were known and novel variants continue to be found (4). An important role of the diagnostic laboratory is to recognise the relatively small number of variant haemoglobins of clinical significance and to distinguish them from the large number of variants that are not clinically significant. Nonetheless, for individual patients the recognition of an unstable or high affinity haemoglobin is an important part of HbA1c analysis as it can lead to misinterpretation of results and inappropriate management that can result in misdiagnosis. HPLC is used as the primary method for HbA1c analysis in many laboratories in New Zealand and an abnormal chromatogram trace does not give specific result of the precise variant, but can elucidate different haemoglobins by means of their physicochemical characteristics, in the context of clinical and family history, ethnic origin, blood count and blood film (4). For this reason it is desirable to always use at least two different laboratory techniques to provide a reasonably reliable identification. Further investigation may include haemoglobin electrophoresis, mass spectrometry studies and DNA analysis (5). This particular patient was found to have a haemoglobin D Punjab (HbD), the replacement of glutamate by glutamine at position β-121 that increases the molecular charge by one so that any glycated HbD co-elutes with HbAo fraction resulting in abnormal peaks that cause interference in the HPLC method (Bio Rad Variant), falsely reducing the HbA1c result. In contrast, the immunoassay (DCA 2000) for HbA1c uses a monoclonal antibody which recognises the glycated N-terminal valine of the β chain and is unaffected by the substitution at position β-121. HbD is a benign variant ubiquitous in the population of India, especially the Punjab region. Variants alter charges on the haemoglobin molecule and give misleading HbA1c results, both higher and lower than the true level of HbA1c (6).

Traditionally, HbA1c measurements were used to monitor long term glycaemic control in patients with diabetes. However, since 2011, according to the New Zealand Ministry of Health, it has become part of current clinical guidelines to endorse HbA1c as a diagnostic marker with concentrations above 50 mmol/mol consistent with diabetes mellitus (7). If patients have a result at or above this level, and hyperglycaemic symptoms are apparent, it confirms the diagnosis. In the absence of symptoms, a repeat HbA1c of >50 mmol/mol confirms the diagnosis as this maximises the specificity of the criteria. In this patient, the HPLC (Bio Rad Variant) result was within the reference interval, however the true result was actually much higher and closer to the diagnostic threshold. In this case, the diagnosis may have been delayed if the laboratory test was being used for that purpose. As such, inspection of the HPLC chromatogram for abnormal peaks is crucial to identify erroneous HbA1c results due to the potential presence of haemoglobin variants.

It is unknown how common haemoglobin variants are in New Zealand, but it is particularly important to have a heightened awareness for their presence when HbA1c is increasingly becoming used as a diagnostic test. Therefore, it is more important than ever to encourage a greater interaction between clinicians and laboratories to identify clinically, but not biochemically, undetectable variants to achieve a valid estimation of glycaemic control in affected patients.
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