Haemoglobin Ypsilanti: an incidental finding in two diabetic patients

Sir,
Haemoglobin Ypsilanti is a rare beta chain haemoglobin (Hb) variant originally diagnosed in a family from Ypsilanti, Michigan, in 1967. This variant is due to a mutation in position 999 where aspartic acid is replaced by tyrosine. As a result of this mutation, haemoglobin molecules have increased oxygen affinity and the clinical manifestation of erythrocytosis may occur. Here, we report two cases of Hb Ypsilanti which were detected as a haemoglobin variant by our HbA1c analyser.

HbA1c measurement was performed using ion-exchange HPLC by Bio-Rad Variant II Turbo 2.0 (Bio-Rad Laboratories, USA). Haemoglobin variant screening was performed by HPLC Bio-Rad Variant II utilising β-thalassaemia short program (Bio-Rad Laboratories). Alkaline and acid gel electrophoreses were performed on Sebia Hydrasys agarose gel electrophoresis system (Sebia Electrophoresis, USA). Full blood count analysis was done on a Sysmex XE-5000 (Sysmex Corporation, Japan).

A 62-year-old Indian female diagnosed with diabetes mellitus (DM) in the year 2002 was regularly followed up for glycaemic control. During the period between 2002 and 2009, HbA1c was measured using immunoassay method (Cobas Integra 800; Roche Diagnostics, Germany) and this method does not detect haemoglobin variants. Average HbA1c and plasma glucose values were 6.5% and 6.5 mmol/L. From 2009 onwards, HbA1c in our laboratory has been analysed by Bio-Rad Variant II Turbo 2.0 (Bio-Rad Laboratories). The presence of variant peak was reported with HbA1c results and the clinician was advised to screen for underlying haemoglobinopathy. The patient’s fasting blood sugar over a period of 3 years ranged from 6.1 mmol/L to 6.9 mmol/L and average HbA1c was 7.5%. Peripheral blood indices were normal except red blood cell count was 4.94 × 10^12/L which showed mild erythrocytosis. A blood sample was sent for Hb analysis on the subsequent visit. Haemoglobin variant screening was performed by HPLC Bio-Rad Variant II utilising β-thalassaemia short program (Bio-Rad Laboratories) and a broad peak measuring 16.6% in the S-window with the retention time of 4.54 min (Fig. 1) was observed. Alkaline and acid gel electrophoreses were performed on Sebia Hydrasys agarose gel electrophoresis system (Sebia Electrophoresis). Electrophoresis at alkaline pH showed the classical appearance of Hb Ypsilanti which was evident by the presence of two prominent variant bands: one between HbA and HbF and the other between HbF and HbS. Acid gel electrophoresis did not show the presence of HbS band (Fig. 2).

The other case diagnosed with Hb Ypsilanti was a 38-year-old Malay male with recent onset of DM who was investigated for glycaemic control. His fasting blood sugar and HbA1c were 5.1 mmol/L and 5.7%, respectively. HbA1c analysis by ion exchange HPLC showed the presence of variant peak. Haemoglobinopathy screening and alkaline gel electrophoresis findings were similar to the previous patient. Haematological parameters were normal for this patient and no erythrocytosis was observed.

HbA1c is used to monitor long-term glycaemic control in patients with diabetes mellitus. The most common Hb variants worldwide are HbS, HbE, HbC, and HbD. Clinically significant interferences with HbA1c measurement have been reported by some methods for each of these variants. Less common Hb variants have also been reported to affect HbA1c measurements. Haemoglobin Ypsilanti is a very rare haemoglobin variant and has not been reported in the context of interference in HbA1c analysis.

Hb Ypsilanti was first diagnosed in 1967 as a high oxygen affinity haemoglobin. The pathophysiological effect of structural alteration in haemoglobin with a high affinity for oxygen is to create a state of relative hypoxia and as a consequence increase in erythropoietin. This causes increase in red blood cell production. The degree of erythrocytosis and the resulting clinical manifestations are highly variable and depend on the degree of altered oxygen affinity. With slight increase in oxygen affinity, the patient is asymptomatic and at most, mildly polycythaemic. In the cases presented in this report, the 62-year-old female had mild erythrocytosis and the 38-year-old male had normal haematological parameters, whereas in the report by Mais et al., a baby presented with polycythaemia and the index case father presented with severe polycythaemia, hyperviscosity and iron deficiency anaemia. The manifestations of the symptoms are due to higher oxygen affinity of this Hb variant. This high affinity for oxygen is responsible for the formation of terameric hybrids in vitro and that appears as two distinct bands on alkaline gel electrophoresis.

Mais et al. found the variant peak migrating in the D-window by Bio-Rad variant β-Thal Short program. However, they also reported that elution time for Hb Ypsilanti by this program may not be specific and recommended performing gel electrophoresis to demonstrate the characteristic variant bands. HPLC analysis of haemoglobin in both our cases showed the presence of variant peak appearing in S-window with the retention time of 4.54 and 4.51, respectively, and gel electrophoresis in alkaline pH demonstrated the presence of characteristic variant bands.

In this part of the world, HbE is a common haemoglobin variant and when this variant is present it would be detected as a variant peak in HbA1c analysis. By looking at the ion exchange HPLC chromatogram it would be difficult to differentiate HbE and less common variants. We have reported in our earlier study that the presence of HbE variant causes positive interference in the measurement of HbA1c. We attributed this possibly to mild overlap of A0 and variant peaks which can

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**Fig. 1** Haemoglobinopathy screening by Bio-rad β-Thal short program. Chromatogram showing a broad variant peak with retention of 4.54 min.
affect the calculation of HbA1c. Since Hb Ypsilanti during HbA1c analysis also appears in the variant window similar to HbE, we postulate that HbA1c levels also may not be accurate in these patients. In the first patient, even though the fasting plasma glucose was stable, the HbA1c value by immunoassay method was lower than ion exchange method. This could be due to interference by Hb Ypsilanti variant in the measurement by ion exchange method. In cases of Hb Ypsilanti presenting with polycythaemia, this could be another contributing factor that may cause falsely increased HbA1c.

The variant Hb Ypsilanti was reported to the referring clinician with a note that HbA1c result may not be accurate. Family studies and DNA analysis have been advised for confirmation of the diagnosis. In conclusion, clinical laboratories and physicians should be aware of the limitations of the HbA1c assay method used in the laboratory. When variant haemoglobin is detected by HbA1c analysis, identification of variants is necessary for a reliable interpretation of HbA1c results. If the variant is known to cause interference in HbA1c assay, other markers such as fructosamine or glycated albumin can be used to assess glycaemic status. Alternatively, HbA1c can be analysed by a method with a different assay principle.

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