CORRESPONDENCE

Effect of HbE trait on measurement of HbA1c by three different methods

Sir,

HbA1c testing is mainly used for monitoring glycaemic control in patients with diabetes. However, the World Health Organization now recommends that HbA1c can be used as a diagnostic test for diabetes, provided that stringent quality assurance procedures are in place and assays are standardised to criteria aligned to the international reference values. Laboratories use many different methods for measuring A1C, but some of these methods can give inaccurate results when the patient has a variant haemoglobin. The effect of common haemoglobin variants (HbS, HbE, HbC and HbD) on HbA1c measurement has been previously studied. Descriptions of the method-specific analytical interferences of these Hb variants on A1C measurement are available on the National Glycohemoglobin Standardization Program (NGSP) website.

In an earlier study performed in our laboratory, a positive bias of ~10% was reported when glycated haemoglobin of patients with haemoglobin (Hb) E trait was measured using Bio-Rad Variant II Turbo 2.0 [cation-exchange high performance liquid chromatography (HPLC) method; Bio-Rad, USA] compared to boronate affinity method. This was attributed to insufficient separation of A0 peak from HbE peak. But recently, Bio-Rad Variant II Turbo 2.0 introduced a new resin lot 11840 and dropline that separates HbA2 from HbA0 in the chromatogram. So it is expected that there should be no interference by the presence of common Hb variants like D and E which fall in the A2 region. Recently enzymatic HbA1c assay has been introduced in the Bio Majestry (BM) 6010/C automated chemistry analyser (JEOL, Japan). In this method, blood cells were first haemolysed, and haemoglobin was digested with protease to yield fructosyl amino acid. Fructosyl amino acid oxidase acts on the fructosyl amino acid and generates hydrogen peroxide, which reacts with chromogens in the presence of peroxidase. Capillary electrophoretic method uses the charge difference between HbA1c and other Hb fractions. Separation is achieved via a high-voltage electrical field and electro-osmotic flow. The Capillaries 2 Flex Piercing instrument (Sebia, France) uses capillary electrophoresis method and it separates normal and abnormal (or variants) levels of haemoglobin in the following order, from cathode to anode: A2/C, E, S, D, F, A0, other haemoglobins (including minor HbA1) and then A1C.

However, few reports are available about the effect of HbE on Hba1c measurement by these three methods. In our routine analysis of HbA1c by the ion exchange method, the most common variant observed is HbE. Hence, we evaluated the effect of HbE trait on the measurement of HbA1c by Bio-Rad Variant II Turbo 2.0 with the new resin lot 11840, Capillaries 2 Flex Piercing for HbA1c, and enzymatic method: BM Test HbA1c on JCA BM 6010/C. Boronate affinity method (Premier HB9210; Trinity Biotech, USA) was used as the comparison method.

Samples that showed the presence of variant window when HbA1c was measured by Bio-Rad variant II turbo 2.0 were collected over a period of 3 months and were stored at −80°C until further analysis. These samples were screened for haemoglobin variant by the automated capillary electrophoresis, using the Capillaries 2 Flex Piercing instrument. A total of 233 samples that were identified as HbE trait by inspecting the chromatogram obtained by capillary electrophoresis and 153 samples of diabetic patients with homozygous A were included for the study.

For each method, results obtained for homozygous A and HbE trait were compared with boronate affinity method. An overall test of coincidence of 2 least-squares linear regression lines was performed using SPSS Software version 18 (SPSS, USA) to determine whether the presence of HbE caused a statistically significant difference (p < 0.05). Method bias was evaluated by Bland–Altman plot and Deming regression

Fig. 1 Deming regression analysis between boronate affinity HPLC and (A) ion-exchange, (B) enzymatic and (C) capillary electrophoresis method.
analysis using Analyse-it method evaluation software (Analyse-it Software, UK). Bias attributable to the presence of Hb variant was studied using /C6 5% [total allowable error by the Royal College of Pathologists of Australasia (RCPA)] at medical decision points of 6% and 9%.

The linearity and assay imprecision for all the four methods were verified in our laboratory. The linearity ranged from 4% to 16% and the assay imprecision was <2%. Good agreement and correlation was observed for homozygous HbA samples between ion-exchange, capillary electrophoresis and enzymatic method with the comparative method, i.e., boronate affinity.

Deming regression analysis for the HbE variant samples by the three methods showed a good correlation with the boronate affinity method (Fig. 1 and Table 1). Statistically significant difference (p < 0.05) was observed for ion-exchange and enzymatic method when compared to boronate affinity method. However, it is not clinically significant at the medical decision point of 6% and 9%. Table 1 lists the differences at 6% and 9% for the three methods. The bias for ion-exchange HPLC, enzymatic and capillary electrophoresis method for the variant samples when compared with the boronate affinity method were 0.2, 0.7 and 0.9%, respectively, which is less than the total allowable error by RCPA external quality assurance (Fig. 2).

Jeppsson et al. reported that different values for HbA1c can be obtained for the same sample by HPLC method depending on the kind of resin, resin lot, column size, buffer composition and elution times.7 Hence, we carried out this study to evaluate whether the change in the resin lot and peak integration in the chromatogram are effective in reducing interference by the presence of HbE trait. In the enzymatic method, glycated dipeptide (fructosyl-valine-histidine) is generated from the N-terminus of the beta-chain of haemoglobin by protease reaction. Since the substitution for glutamic acid by lysine is at position 26 of the b-globin chain in HbE, there should be no interference in the measurement of HbA1c by enzymatic method. Similar to the findings of Matsumoto et al., we also observed that BM Test HbA1c on JCA BM 6010/c is not affected by the presence of HbE trait. The non-glycated variant peak of HbE is well separated from A1C and A0 peak by capillary electrophoresis. Even though the glycated fraction of HbE is probably confounded with HbA0 peak, studies have shown that it does not interfere with HbA1c measurement.3,4 In the present study, none of the three methods evaluated showed clinically significant effects due to the presence of HbE trait. HbA1c measurement by Bio-Rad Variant II Turbo 2.0 did not show clinically significant interference due to the presence of HbE trait. This is probably due to the changes in the peak integration of A0 from A2 and also due to different resin lot. In choosing the method, laboratory personnel must be aware of the effect of locally prevalent haemoglobin variant on the measurement of HbA1c; more so if HbA1c is used for the diagnosis of diabetes mellitus.

Table 1

<table>
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<th>Method</th>
<th>HbA1c MDP</th>
<th>Bias</th>
<th>95% CI</th>
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<tr>
<td>Bio-Rad Variant II Turbo 2.0</td>
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<td>0.02 to 0.06</td>
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<tr>
<td>Capillary electrophoresis</td>
<td>6%</td>
<td>0.04</td>
<td>0.01 to 0.06</td>
</tr>
<tr>
<td>Enzymatic method</td>
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<td></td>
<td>9%</td>
<td>0.01</td>
<td>-0.03 to 0.05</td>
</tr>
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Arjoanna Azizi1
Pavai Sthaneshwar1
Hemalatha Shannugam1
Shannuganathan Arumugam2

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Table 1 Details the differences at 6% and 9% for the three methods. The bias for ion-exchange HPLC, enzymatic and capillary electrophoresis method for the variant samples when compared with the boronate affinity method were 0.2, 0.7 and 0.9%, respectively, which is less than the total allowable error by RCPA external quality assurance. Fig. 2 shows the Bland–Altman Plot between Premier Hb9210 and (A) Bio-Rad Variant II turbo 2.0, (B) enzymatic assay (Bio Majesty (BM) 6010/c) and (C) Sebia capillary electrophoresis method.
Contact Pavai Sthaneshwar.
E-mail: pavai@ummc.edu.my

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