

## Short reports

Haemoglobin O Padova and falsely low haemoglobin A<sub>1c</sub> in a patient with type I diabetes

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## Abstract

Glycated haemoglobin (HbA<sub>1c</sub>) measured by high performance liquid chromatography (HPLC) in a 20 year old female with insulin dependent diabetes mellitus was consistently within the normal range although her daily blood glucose values were > 11.1 mmol/l. HbA<sub>1c</sub> measured by immunoagglutination and fructosamine was elevated and correlated with the patient's blood glucose values. The HPLC chromatogram showed an additional peak at HbA<sub>o</sub>. Electrophoresis of haemoglobin on citrate agar gel revealed an abnormal haemoglobin anodal of HbS. Cellulose acetate electrophoresis and isoelectric focusing demonstrated an additional haemoglobin migrating close to HbA<sub>2</sub>. Amino acid analysis and DNA sequencing revealed an  $\alpha$  30 (B11) Glu→Lys replacement, that is, haemoglobin O Padova. Investigations of two family members without diabetes revealed the same rare haemoglobin variant. This case showed that this silent haemoglobin mutation caused an additional peak and falsely low HbA<sub>1c</sub> values when measured by HPLC, the gold standard for this evaluation. (J Clin Pathol 1997;50:434-435)

Keywords: high performance liquid chromatography; haemoglobin variants; insulin dependent diabetes mellitus

A 20 year old white female patient with insulin dependent diabetes mellitus treated with insulin, presented to the diabetes outpatient clinic of the department of internal medicine at Karl Franzens University, Graz, Austria. The glycated haemoglobin (HbA<sub>1c</sub>) measurement of 6.2% measured with high performance liquid chromatography (HPLC) (Diamat, Bio-Rad, Vienna, Austria) was just above the upper limit of normal (4.2-6.1%) (fig 1) and suggested good blood glucose control. Her mean blood glucose concentration was elevated at 12.4 mmol/l (mean of four to five daily home measurements during one week before HbA<sub>1c</sub> evaluation), her HbA<sub>1c</sub> measured by latex immunoassay (DCA 2000, Bayer, Vienna, Austria) was 8.9% (normal 4.5-5.7%), and serum fructosamine was also elevated to 398  $\mu$ mol/l (normal < 285  $\mu$ mol/l).

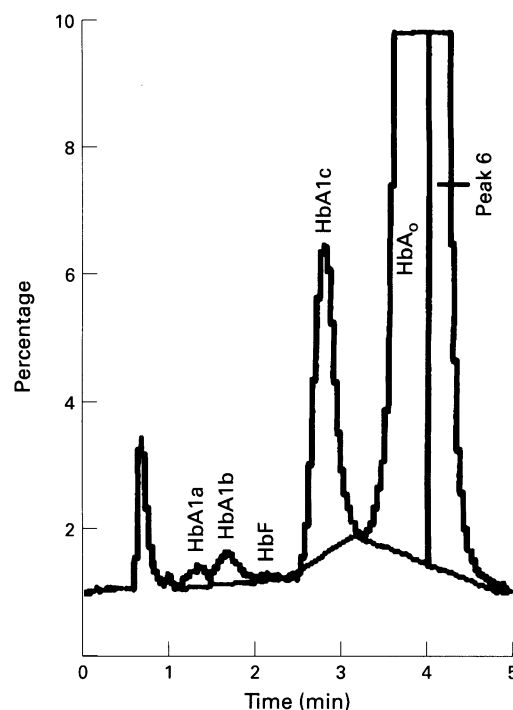


Figure 1 HPLC chromatogram and haemoglobin report of a patient with insulin dependent diabetes mellitus with haemoglobin O Padova. HbA<sub>1a</sub>, 0.4%; HbA<sub>1b</sub>, 0.6%; HbF, 0.1%; HbA<sub>1c</sub>, 6.2%; HbA<sub>2</sub>, 73.1%; peak 6, 19.7%.

Investigation of two family members without diabetes revealed haemoglobin O Padova values of 4.7% with HPLC, and 5.3% and 5.2% with immunoagglutination. Their serum fructosamine was in the normal range. Routine haematological indices and all other parameters were normal in all three family members.

Electrophoretic analysis of a blood sample from the patient with diabetes on cellulose acetate membranes (Helena Laboratories, Beaumont, Texas, USA) using Tris EDTA borate buffer at pH 8.4<sup>1</sup> revealed an abnormal haemoglobin migrating very close to HbA<sub>2</sub>, the two variants accounting for 24% of total haemoglobin.<sup>2</sup> Citrate agar electrophoresis of haemoglobin using citrate buffer at pH 6.2 demonstrated an additional haemoglobin slightly anodal of HbS.<sup>3</sup> Isoelectric focusing on commercial gel plates revealed a haemoglobin close to HbA<sub>2</sub>. Quantification of HbA<sub>2</sub> with a cation exchange chromatography kit (Haemoglobin Testing System,  $\beta$ -Thalassemia Short

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Program, Bio-Rad) showed 1.8% HbA<sub>2</sub> and 20.2% abnormal haemoglobin. Amino acid analysis of isolated tryptic peptides and sequence analysis of amplified DNA<sup>4</sup> revealed an  $\alpha$  30 (B11) Glu→Lys mutation identical to that described for the silent haemoglobin variant O Padova.<sup>2,5</sup> A heat stability test did not reveal unstable haemoglobins. HbF and the intracellular distribution of HbF were normal. Heinz bodies and HbH inclusion bodies<sup>4</sup> were not found.

### Conclusions

We report the case of a 20 year old female patient with insulin dependent diabetes and two of her family members, all bearing the rare silent haemoglobin variant O Padova. The haemoglobin variant caused falsely low HbA<sub>1c</sub> measurements and an additional peak in the HPLC chromatogram (fig 1) in the patient with diabetes.

There are several methods available for HbA<sub>1c</sub> determination.<sup>6</sup> HPLC with cation exchange columns was recently selected to be the reference for HbA<sub>1c</sub> measurement with which other methods are to be compared.<sup>7</sup> Using this HPLC method, the O Padova peak seems to be included in the total peak calculation, thereby falsely lowering the HbA<sub>1c</sub> value.

Periodic measurement of HbA<sub>1c</sub> validates the accuracy of home blood glucose measurements of patients with intensified insulin therapy. The Diabetes Control and Complications Trial predicted an increase in the number of glycohaemoglobin tests.<sup>8</sup> A deviation of 1% in HbA<sub>1c</sub> reflects a change of about 1.4–1.9 mmol/l in average blood glucose; therefore, HbA<sub>1c</sub> is highly sensitive to blood glucose elevations. Glycohaemoglobin more than 3% above the normal limit indicates mean blood glucose levels of more than 11.1 mmol/l during the three months before investigation.<sup>6</sup> Other factors that can alter the charge of haemoglobin and influence glycohaemoglobin results include uraemia,<sup>9</sup> alcohol dependence,<sup>10</sup> and high doses of aspirin.<sup>11</sup> HbF levels are elevated in some diabetic patients and this may lead to inappropriately high HbA<sub>1c</sub> results.<sup>12</sup>

If the clinical impression and HbA<sub>1c</sub> results do not match in the case of repeatedly inappropriate HbA<sub>1c</sub> or additional peaks in HPLC chromatograms, the HbA<sub>1c</sub> values must be determined with a second method such as immunoagglutination.<sup>13</sup> Blood glucose measurements and fructosamine results are not always well correlated,<sup>14</sup> therefore, correct HbA<sub>1c</sub> values and mean blood glucose are the most appropriate methods, but only if the contribution of variant haemoglobins are properly accounted for.

HPLC has been shown to separate several haemoglobin variants. Some variants cause excessively high HbA<sub>1c</sub> values.<sup>15</sup> Haemoglobinopathies such as haemoglobin C and S may falsely lower glycohaemoglobin values.<sup>16</sup>  $\alpha$  chain haemoglobin variants lead to either under or over estimation of HbA<sub>1c</sub>,<sup>17</sup> and may cause additional single peaks or, as in the present report, a peak that overlaps with the HbA<sub>0</sub> peak.

We conclude that haemoglobin O Padova can contribute to mismanagement of patients with insulin dependent diabetes mellitus because of falsely low HbA<sub>1c</sub> measured by HPLC.

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