



Normal glycated haemoglobin in a patient with poorly controlled diabetes mellitus and haemoglobin D Punjab: implications for assessment of control

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A 52-year-old male patient was being monitored for control of type I diabetes mellitus with regular checks of his glycated haemoglobin (HbA_{1c}). The values obtained were unexpectedly low and in practise would only be obtainable at the expense of frequent hypoglycaemia. Moreover, they appeared inconsistent with the plasma glucose values (Table 1).

In September 1998, a new high performance liquid chromatography (HPLC) (BioRad Variant) analyser revealed a double haemoglobin A (HbA_o) peak not previously recognised by an older HPLC method. This prompted further studies. Haemoglobin was purified by ion exchange chromatography and further analysed by electrospray ionization mass spectrometry (ESI-MS). This revealed normal alpha chains (molecular weight 15126 Da) but abnormal beta chains (15866 Da), a decrease of 1 Da. Examination of tryptic digests of purified abnormal globin by ESI-MS showed that the observed 1 Da mass difference was in the peptide β -13, and could be accounted for by a substitution of glutamine for glutamate at amino acid 121. This occurred in about half of the beta chains, indicating heterozygosity for a recognised amino acid substitution, haemoglobin D Punjab.¹

Further specimens were analysed in tandem by HPLC as well as by an immunological method (DCA 2000 – Bayer) which is not affected by variant haemoglobins. As expected, the DCA 2000 results were higher (though still showing reasonable control), reflecting the prevailing glucose levels (Table 1).

Table 1. Glucose and HbA_{1c} values by different methods in a patient with diabetes mellitus and Hb D Punjab. *Target range <7% for both methods.

Sample	Glucose (mmol/L)	HbA _{1c} by HPLC method (%)*	HbA _{1c} by DCA 2000 (%)*
1	15.9	4.3	-
2	11.2	4.0	-
3	13.0	4.6	-
4	15.0	4.5	6.7
5	15.0	4.5	7.2

Discussion

The Variant HbA_{1c} method uses cation exchange HPLC to separate various haemoglobin fractions. A buffer gradient of increasing ionic strength elutes any haemoglobin fractions adhering to the cation exchange particles in order of increasing net positive charge on the haemoglobin molecules. The addition of glucose to the N-terminal of the beta chains in HbA_{1c} reduces net positive charge by 1 so that HbA_{1c} elutes from the cation exchange column ahead of HbA_o.

In Hb D Punjab, the replacement of glutamate by glutamine at position β -121 increases the molecular charge by one so that any glycosylated HbD co-elutes with HbA₀. This therefore reduces the apparent value for total glycosylated haemoglobin. In contrast, the immunoassay for HbA_{1c} uses a monoclonal antibody which recognizes the glycosylated N-terminal four amino acids of the beta chain and is unaffected by the substitution at position β -121.

HbD Punjab is in itself an otherwise benign variant, common in India especially the Punjab. There are over 700 known haemoglobin variants, many of which alter the charge on the haemoglobin molecule and give misleading results, both higher and lower than the true level.² Other causes of misleading results, depending on the analytical method used, include haemolytic anaemia (by shortening red cell survival), uraemia, lead poisoning, alcoholism, high dose salicylates and hereditary persistence of foetal haemoglobin.³

With the increased incidence of haemoglobin variants as a consequence of immigration, doctors need to be aware that misleading HbA_{1c} levels will become more frequent. As with other laboratory tests, it would be appropriate to communicate with the laboratory service when results are not in keeping with the clinical picture.

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