CASE REPORT

Haemoglobin Marseille-Long Island and interpretation of HbA1c: which HbA1c result is the "right answer"?

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A woman was screened for diabetes using glycated haemoglobin (HbA1c). Vastly different results were obtained by high performance liquid chromatography (45%), immunoassay (2.9%), and affinity chromatography (4.2%) compared with the non-diabetic range of less than 6.4%. Mass spectral studies confirmed the presence a haemoglobin variant, haemoglobin Marseille-Long Island which had confounded interpretation by all methods.

An asymptomatic 44 year old woman was screened for diabetes using glycated haemoglobin (HbA1c), an index of average glycaemia, during the preceding 1-3 months.¹

Initial HbA1c analysis was undertaken by high performance liquid chromatography (HPLC) on the Bio Rad Variant (Bio Rad Laboratories Inc, California, USA) and gave a result of 45% (non-diabetic range up to 6.4%). This result was considered to be biologically implausible and HbA1c analysis was therefore undertaken by other analytical methods. HbA1c was therefore analysed by immunoassay on the DCA 2000 instrument (Bayer Diagnostics) and was 2.8%, which was considered to be implausibly low.

It was decided to arrange further HbA1c analysis by affinity chromatography on the Primus instrument (Primus Corporation, Kansas City, MO, USA) which gave a result of 4.6%. Haemoglobin was submitted to mass spectrometry (VG Platform; Micromass, UK) which confirmed the presence a haemoglobin variant, identified as haemoglobin Marseille-Long Island (see fig 1) which confounds interpretation of these analytical methods.

DISCUSSION

The initial HbA1c of 45% by HPLC is implausibly high, especially in an asymptomatic woman with a low probability of diabetes. This raised suspicion of a possible haemoglobin variant. Haemoglobin electrophoresis on cellulose acetate (pH 8.6) gave a normal pattern. This prompted the decision to undertake mass spectrometry studies of HbA1c.

Mass spectrometry excluded a HbA1c of 45% and confirmed that the subject was heterozygous for haemoglobin Marseille-Long Island. This variant, originally termed haemoglobin Marseille² was originally described in a diabetic Maltese woman, and subsequently termed haemoglobin Long Island.³ Structural analysis revealed the presence of a methionyl residue extending the amino terminus of the β globin chain and a histidine to proline substitution at the second position in the β chain.² This substitution of a positively charged amino acid with a neutral one results in a net loss of one positive charge. Haemoglobin Marseille-Long Island therefore has lower affinity for the ion exchange resin and coelutes with the HbA1c peak resulting in an artefactually high reading.

The immunoassay method (DCA 2000) relies on the ability of a specific antibody to recognise glucose bound to the amino

Figure 1 Mass spectral analysis of control and patient lysate showing variant β chain (β^{Mar}) with a mass increase of 91 Da. This histidine to proline substitution decreases the mass by 40 Da and the addition of N terminal methionine results in a mass increase of 131.

terminal amino acids of the β globin chain.⁴ In the present case, the β globin chains from haemoglobin Marseille-Long Island have an amino terminal that is modified in a way that does not permit recognition by the antibodies of the DCA 2000 assay. Given that the subject is heterozygous for this variant and that half the β chains are therefore normal, then arguably the "correct" answer might be obtained by doubling the DCA 2000 result. This would give a value of 5.8%, which is plausible.

Affinity chromatography relies on a fundamentally different analytical principle, namely a covalent interaction between cis-diol groups in the glycated haemoglobin molecule (at all sites and not just the amino terminus of the β chain) and boronate residues in the affinity resin. Results are usually adjusted to give HbA1c equivalents⁵ and this method is the one normally considered to be least affected by haemoglobin variants. The modification of the amino terminus of the β globin chain in haemoglobin Marseille-Long Island, in particular the methionyl extension means that the fraction that is technically HbA1c does not exist. The absence of glycation at this site results in a value that is artefactually low. In the absence of any other evidence, this result, which is biologically plausible might have been accepted as "correct" in the present case.

Abbreviations: HbA1c, glycated haemoglobin; HPLC, high performance liquid chromatography

Learning points

- Haemoglobin variants can interfere with most HbA1c methods and cause problems with interpretation.
- The possibility of haemoglobin variants should be considered when HbA1c results do not concur with clinical expectations.
- Haemoglobin variants may not always be revealed by electrophoresis.
- Analysis of HbA1c by alternative methods, in particular by mass spectrometry may help to elucidate the nature of confounding variants.
- It is occasionally necessary to consider alternative measures of glycation than HbA1c.

None of the obtained results therefore gives the “right answer” for glycaemic status. As indicated above, doubling of the DCA 2000 result may be argued as giving the “right answer”. Otherwise, an alternative measure of glycation such as fructosamine⁶ should be employed. It is also possible to use mass spectrometry (with proteolytic digestion and quantitation of N terminal glycated peptides) as a method for HbA1c estimation. There is a strong view, supported by the International Federation of Clinical Chemistry, that it should be adopted as a reference method on account of its specificity.⁷ Measurement of HbA1c by mass spectrometry in the present case would have been misleading given that half of the β chains show methionyl extension.

Other methods are not specific for HbA1c, although are traceable to the HPLC method (Biorex 70) that was used in the Diabetes Control and Complications Study,⁸ and gave higher values. Opponents of the International Federation of Clinical Chemistry position favour that HbA1c methodologies should be aligned to the method employed in the Diabetes Control and Complications Study trial.⁷ The debate continues.

Notwithstanding all of the above considerations, the predictive value of HbA1c for diabetes depends on the chosen cut off and is not usually recommended as a screening test.⁹

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