

A patient with hyperglycaemia and normal HbA_{1c} due to impaired glycation

M.J.M. Diekman^{1*}, H.J.M. Salden², J.H. DeVries³

Departments of ¹Internal Medicine and ²Clinical Chemistry, Deventer Hospital, Deventer, the Netherlands, ³Department of Internal Medicine, Academic Medical Centre, Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)570-64 66 66, e-mail: m.j.m.diekman@dz.nl

ABSTRACT

A diabetic Caucasian woman presented with discrepantly low HbA_{1c} values compared with her glycaemia. High-performance liquid chromatography (HPLC) analysis disclosed 80% HbA and 20% HbI Philadelphia (16α2 lys → glut). The calculated glycosylation gap from the fructosamine level was 1.2%. The haemoglobin α/β glycation ratios, as measured by electron spray ionisation mass spectroscopy (ESI-MS), for the patient and her three children also carrying the mutation were decreased by values of 0.56 and 0.51, 0.50 and 0.49, respectively (reference value 0.66).

KEYWORDS

Haemoglobinopathy, Hb Philadelphia, HbA_{1c}, glycation

INTRODUCTION

The measurement of glycohaemoglobin (glyHb or HbA_{1c}) serves as a powerful tool in the evaluation and management of patients with diabetes mellitus. In 1969, Rahbar accidentally observed the presence of an increased percentage of glycohaemoglobin (glyHb or HbA_{1c}) in the blood of diabetic patients.¹ Trivelli used this phenomenon as an indicator of long-term glycaemic control as it reflects blood glucose levels over the preceding six to eight weeks.² Subsequently, this measurement was adopted by clinicians worldwide. HbA_{1c} levels correlate well with the risk of development of chronic complications.^{3,4} Mean HbA_{1c} levels of a hospital or practice are increasingly being used to assess quality of care and as a benchmark parameter. Knowledge of sources of variations in HbA_{1c}, analytical or biological, is needed for correct interpretation.

Glycohaemoglobin is formed *in vivo* by the irreversible attachment of glucose to haemoglobin, a stable post-translational modification. Erythrocytes are freely permeable for glucose which can form an adduct to the N-terminal amino acid valine of the β chain of the haemoglobin molecule. The formation is nonenzymatic and the rate depends essentially on the ambient glucose concentration.⁵

There are some well-known caveats when interpreting HbA_{1c} values. A normal HbA_{1c} can give a false sense of reasonable control when in real life, blood glucose concentrations fluctuate greatly. HbA_{1c} concentration also depends on the erythrocyte lifespan as more HbA_{1c} is formed with longer exposure to glucose. Outside the context of classic haemoglobinopathies causing chronic haemolysis, structural varieties in the haemoglobin molecule can also lead to spurious laboratory results due to altered affinity for glucose. An example of this from daily practice will be presented here.

CASE REPORT

A 77-year-old woman presented with a consistently discrepantly low HbA_{1c} (5.8%) compared with her fasting blood glucose levels (*table 1*). A random glucose day curve revealed a fasting blood glucose of 14.2, a postprandial value of 13.2 and a pre-dinner value of 8.2 mmol/l. For the last 20 years she had suffered from diabetes mellitus complicated by a mild nonproliferative retinopathy. Her current treatment consisted of a carbohydrate spread diet and metformin 500 mg/daily and glibenclamide 5 mg three times/day. Other medication included verapamil 240 mg/daily and aspirin 80 mg/daily. On physical examination her weight was 56 kg, height 1.55 m, body

Table 1. Review of fasting blood glucose and HbA_{1c} values in the years before presentation

Years before presentation	5	4	3	2	1	0
Fasting glucose (mmol/l)	11.1	10.1	13.8	12.8	12.6	14.2
HbA _{1c} (%)	4.0	6.0	5.8	6.0	5.7	5.8

mass index 23 and office blood pressure 160/80 mmHg. Protective sensibility of her feet was intact.

Laboratory investigations showed neither anaemia nor haemolysis (Hb 8.4 mmol/l, reticulocytes 106/nl, bilirubin 11 µmol/l, lactate dehydrogenase 343 U/l and haptoglobin 1.3 g/l) and normal renal function (creatinine 84 µmol/l and albuminuria 6 mg/24 h). Continuous 24-hour blood glucose monitoring ruled out hypoglycaemia and showed a mean blood glucose concentration of 9.0 mmol/l (figure 1). Fructosamine was 318 µmol/l (reference range 191-288 µmol/l, measured with a spectrophotometric assay (nitroblue tetrazolium (NBT) assay, Roche®).

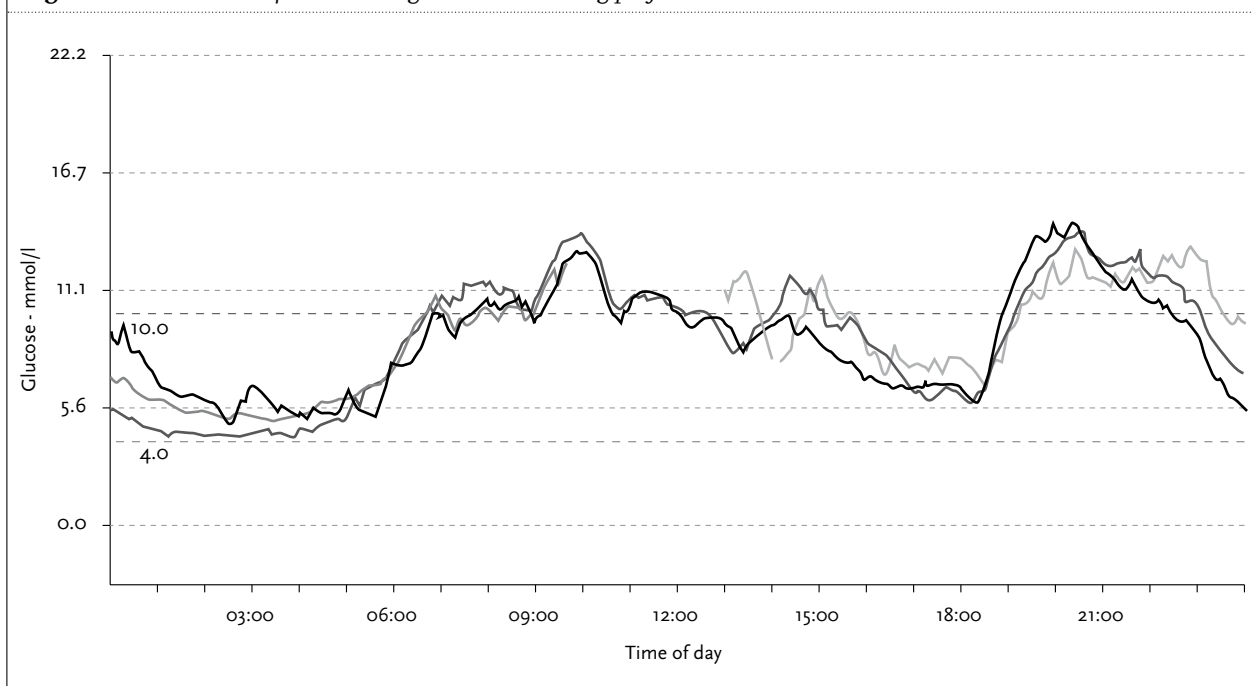
HbA_{1c} measured with a routine turbidimetric immunoassay (Tina-quant, Roche®) and with high-performance liquid chromatography (HPLC) resulted in comparable values, but the latter method disclosed a haemoglobin variant consisting of 80% HbA and 20% HbI. DNA sequencing showed an AAG → GAG transition on codon 16 of the α2 gene leading to a substitution of lysine by a glutaminic acid molecule (α 16 lys → glut, HbI Philadelphia). Three nondiabetic children of the patient were heterozygous for HbI Philadelphia and had fasting glucose concentrations (and HbA_{1c} %) of 5.4 mmol/l (5.4%), 4.9 (5.2%) and

5.1 (5.5%) respectively. The α/β glycation ratios for the patient and her three children measured by electron spray ionisation mass spectroscopy (ESI-MS) were 0.56 and 0.51, 0.50 and 0.49, respectively (reference value 0.66).⁶

DISCUSSION

HbA_{1c} is a stable minor haemoglobin variant originally identified by separation of haemoglobin using cation exchange chromatography. It is mainly composed of glycohaemoglobin primarily glycated at the valine (position 1) of the N-terminal β-chain. However other amino-acids in the haemoglobin molecule can bind glucose. The ε-amino group of lysine at position 16 on the α chain is the second preferred site for glycation. This patient's haemoglobin is mutated precisely at this position where a lysine is exchanged for a glutaminic-acid residue, thus reducing the possible glycation sites which leads to less glycation. Glycation can be measured separately at the α and β chains by electron spray ionisation mass spectroscopy (ESI-MS). The mean α/β glycation ratio was 0.66 in a large group (n=1022) of diabetic patients.⁶ Both

Figure 1. Continuous 24 hour blood glucose monitoring profiles



the patient and her three children have α/β glycation ratios lower than 0.66 suggesting an effect of the mutation on the glycation process.

HbA_{1c} values have a considerable biological variation when tested in patients with a comparable rate of glycaemic control suggesting the existence of slow and rapid glycation.^{7,8} Cohen *et al.* studied this phenomenon and developed a measure of discordance between the actually measured and the predicted HbA_{1c} values from fructosamine (HbA_{1c} = 0.017 x fructosamine (uM) + 1.61): the glycosylation gap.⁹ Applying this formula, the predicted HbA_{1c} value of the discussed patient is 7.0%. With a measured value of 5.8% the resulting gap amounts to 1.2% pointing to impaired glycation.

The low HbA_{1c} value in our patient is not explained by anaemia or haemolysis but there might be a shorter lifespan of erythrocytes containing the mutant haemoglobin, aggravated by hyperglycaemia, which is not expressed in the routine clinical parameters of haemolysis.¹⁰ In theory a chemical interference of the glycation process of the mutant haemoglobin by medication is possible. *In vitro* and animal studies report inhibition of glycation by metformin and aspirin.^{11,12} Her three children carrying the haemoglobin variant have seemingly normal HbA_{1c} values in agreement with their normal blood glucose levels. Why don't they have lower HbA_{1c} values? Maybe glycation of the haemoglobin variant proceeds normally with euglycaemia but is slower when hyperglycaemia is present (glycaemia-dependent kinetics).¹³

When Hb Philadelphia leads to impaired glycation, a difference in the concentration of HbA_{1c} can be expected between the amount measured by an immunometric method, which specifically measures glycation at the N-terminal valine of the β chain, and the amount measured by HPLC, measuring total glycation on both α and β chains. This small difference is, however, not detectable with routine assay methods. The rate of glycation can be measured experimentally, e.g. as reported in the case of the haemoglobin variant Hb Görwihl ($\alpha_2\beta_2$ 5 (A2) Pro \rightarrow Ala) which exhibits impaired glycation.¹⁴ However, experimental protocols are neither well validated nor widely available. In this elderly woman, glycaemic control was guided by serial blood glucose measurements with priority given to avoiding hypoglycaemia. The dose of the long-acting glibenclamide was halved and small doses of short- and rapid-acting insulin were given with breakfast and dinner.

In conclusion, whereas general treatment guidelines recommend to aim for HbA_{1c} values of 7.0% or lower, individual patient management demands targets to be tailor-made. Physicians should be aware of the fact that spuriously low HbA_{1c} values can be caused by clinically silent haemoglobinopathies.

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