

CASE REPORT

Artefactually low glycated haemoglobin in a patient with severe hypertriglyceridaemia

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This report describes a case of artefactually low glycated haemoglobin (Hb) in a patient with type II diabetes and severe hypertriglyceridaemia. The effect of hypertriglyceridaemia on glycated Hb determination using the Abbott Vision method was investigated in a series of patients with diabetes. The interference of triglycerides in glycated Hb assays was also investigated by two other methods, the Beckman Synchron CX4 delta immunoturbidimetric method, and the Primus affinity chromatography high performance liquid chromatography assay.

The patient was a 50 year old woman who smoked about 10 cigarettes each day and drank alcohol only occasionally. She had type II diabetes mellitus, which had been diagnosed approximately 10 years previously. She was also known to have coronary artery disease and had previously undergone a cholecystectomy. Over the past five years she had suffered six attacks of acute pancreatitis necessitating hospital admission. There was a family history of hyperlipidaemia, diabetes mellitus, and coronary artery disease on the paternal side. She was seen in the diabetic clinic for a routine follow up appointment when it was reported that her clinic visit glycated haemoglobin (Hb) was 4.8% (non-diabetic reference range, 4.1–5.7%). However, the clinic doctor doubted this result because her home glucose monitoring (using Roche Diagnostics BM-Test 1–44 strips for blood glucose monitoring) showed blood glucose concentrations consistently between 11 and 18 mmol/litre. Her medication at this time consisted of human mixtard insulin (about 30–40 units twice daily, depending upon her blood glucose results), aspirin 75 mg once daily, and fenofibrate 200 mg (micronised) once daily. The glycated Hb had been measured in the clinic on an Abbot Vision analyser (Abbot Diagnostics, Maidenhead, Berkshire, UK).

Her random serum cholesterol was 11.6 mmol/litre and triglycerides were 27.3 mmol/litre assayed on a Vitros 950 biochemistry analyser (Ortho-Clinical Diagnostics, Amersham, Buckinghamshire, UK).

In view of the apparent discrepancy between the clinic glycated Hb result and her home finger prick blood glucose readings an interference problem with the glycated Hb was suspected.

The glycated Hb was repeated after separating and discarding the plasma and washing the blood cells twice in normal saline before assaying. The glycated Hb of the washed red blood cells was 12.2%, more in keeping with her diabetic control and home blood glucose results.

To confirm that severe hypertriglyceridaemia was the explanation for the spuriously low glycated Hb measured by the Abbott Vision analyser, we subsequently reviewed the glycated Hb on 98 patients with type II diabetes and random serum triglyceride concentrations > 5 mmol/litre (mean, 25.5 mmol/litre; SD, 8.3; range, 5.2–62.0). Where the triglyceride

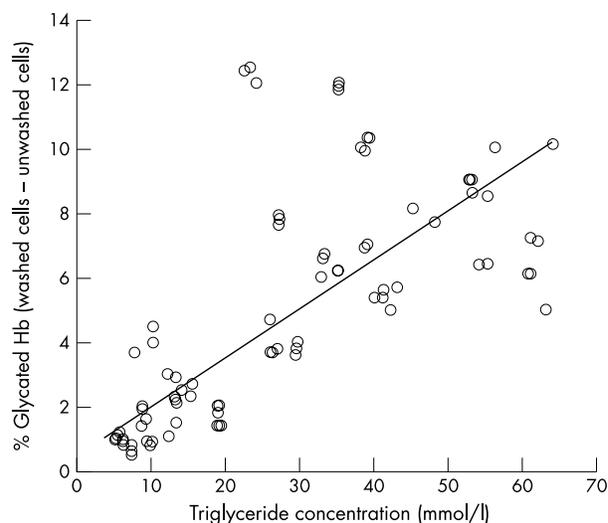


Figure 1 Delta % glycated haemoglobin (Hb) measured by the Abbott Vision method versus triglyceride concentration. Glycated Hb was measured on whole blood samples for 98 patients before and after washing with phosphate buffered saline. The differences in values of washed red blood cells minus unwashed cells were then plotted against triglyceride concentrations.

concentration was > 15 mmol/litre there was a significant increase in glycated Hb in the washed compared with the unwashed red blood cells (mean, 10.4%; SD, 2.9% v mean, 6.4%; SD, 2.2%; $p < 0.0001$, Wilcoxon matched pairs test).

A correlation plot of the serum triglyceride concentration versus the difference in glycated Hb of the washed and unwashed red cells had a Spearman correlation coefficient of 0.95 ($R^2 = 0.51$; 95% confidence interval, 0.056 to 0.120; $p < 0.001$) (fig 1).

We also investigated triglyceride interference with the glycated Hb assay using two other methods: the Beckman Synchron CX4 delta immunoturbidimetric method and the Primus affinity chromatography high performance liquid chromatography assay (HPLC). To compare these methods with the Abbott Vision method we assayed glycated Hb before and after washing with saline in 13 samples from patients with diabetes who had severe hypertriglyceridaemia (triglycerides, 15–60 mmol/litre). The difference in glycated Hb values obtained before and after washing was calculated and subjected to a Spearman analysis, which gave the highest positive correlation ($R^2 = 0.65$; $p < 0.005$) for the Abbott Vision method. With the Beckman Synchron and the Primus

Abbreviations: Hb, haemoglobin; HPLC, high performance liquid chromatography

methods the correlations were not significant ($R^2 = 0.007$; $p = 0.79$) and ($R^2 = 0.018$; $p = < 0.66$), respectively.

DISCUSSION

The Abbott Vision glycoated Hb test is an affinity chromatography assay standardised to haemoglobin A1c using individual affinity chromatography cartridge devices.¹ Abbott claim in their method literature that triglyceride concentrations < 14.7 mmol/litre (1300 mg/dl) show less than 10% interference.² However, they do not indicate the severity of the possible interference at triglyceride concentrations above 14.7 mmol/litre that we have found.

"We suggest that the Abbott Vision method suffers from a turbidity effect of triglyceride, increases both absorbances, and therefore decreases the calculated percentage glycoated haemoglobin

Grossly lipaemic samples are known to present problems to laboratories when assaying certain analytes. For example, pseudohyponatraemia is well documented with flame photometry and non-direct, ion selective electrodes for the assay of serum or plasma sodium.³ Serum amylase results can also be falsely low in patients with severe hypertriglyceridaemia. This is a particular concern because patients with hypertriglyceridaemia are more at risk of acute pancreatitis.^{4,5} Other potentially misleading laboratory abnormalities in the presence of severe hypertriglyceridaemia include hyperbilirubinaemia, increased haemoglobin concentrations,⁶ and decreased arterial oxygen saturation.^{7,8} We suggest that the Abbott Vision method suffers from a turbidity effect of triglyceride, which increases the absorbances of the total Hb and unbound Hb fractions, therefore decreasing the calculated percentage glycoated Hb. However, in the Beckman Synchron CX4 delta immunoturbidimetric and the Primus affinity chromatography HPLC methods the turbidity and the glycoated Hb absorbances are separated by the liquid chromatography.

We conclude that lipaemic blood samples can cause spuriously low glycoated Hb results in patients with diabetes by the Abbott Vision method with potentially serious clinical implications. In our case, the spuriously low glycoated haemoglobin could have been interpreted as showing a risk of manifesting hypoglycaemic episodes. In reality, her glycoated Hb was high, reflecting poor glycaemic control. In contrast, the Beckman Synchron and Primus methods were not affected by severe hypertriglyceridaemia. The Abbott Vision glycoated haemoglobin assay is not widely used by clinical laboratories in the UK. However, it is important to report the limitation of

Take home messages

- Lipaemic blood samples can cause spuriously low glycoated haemoglobin (Hb) results in patients with diabetes by the Abbott Vision method, which could have serious clinical implications
- The Beckman Synchron and Primus methods are not affected by severe hypertriglyceridaemia
- Although the Abbott Vision glycoated haemoglobin assay is not widely used by clinical laboratories in the UK, clinicians and laboratory staff should be aware of potentially spurious results with lipaemic samples

this method to clinicians and laboratory staff. Clinicians should be aware of potentially spurious results with lipaemic samples.

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