

Pitfalls of Glycated Hemoglobin in the Glycemic Assessment of Diabetes Patients with Hemoglobin Louisville: Role of Serum Fructosamine

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Introduction

Measurement of glycated hemoglobin (HbA1c) is widely used in the management of patients with diabetes mellitus (DM). We report on three patients with a history of type 2 DM and consistent elevation of fasting blood glucose values, but with falsely low HbA1c values.

Fasting blood glucose measurements were made using the hexokinase method in a Cobas 6000 (Roche Diagnostics, Rotkreuz, Switzerland). Determinations of HbA1c were performed on ethylenediaminetetraacetic acid–anticoagulated blood sample by two methods: high-performance liquid chromatography (HPLC) (HA-8160 in diabetic mode, A. Menarini Diagnostics, Florence, Italy), which detected no abnormal hemoglobin (Hb); and immune-turbidimetric method (Tina-quant Hemoglobin A1c Gen3 in a Cobas 6000), which was used for two patients. Both methods were calibrated according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Reference Measurement Procedure. Results are shown in **Table 1**.

All three patients had evidence of hemolytic anemia, and their average Hb levels were as follows: patient A: 9.41 g/dl, patient B: 10.9 g/dl, patient C: 8.6 g/dl. Abnormally low HbA1c concentrations are usually encountered in patients with high turnover rates of hemoglobin.¹ Further investigations revealed that the patients belonged to the same family, and all three had a history of heterozygosity trait for Hb Louisville.² Hemoglobin Louisville is an unstable Hb that differs from HbA by the substitution of a phenylalanine residue for a leucine residue in position 42 of the β globin chain, resulting in instability in the Hb molecule, with increased erythrocyte destruction, and clinically manifested as hemolytic anemia². The Hb Louisville heterozygotes suffer from mild anemia, jaundice, and hemolytic crisis.

Upon testing our patients, the abnormal Hb could not be separated from normal HbA by electrophoresis method,³ but interference in the HbA2 zone was noted using the HPLC Bio-Rad D-10TM Dual Program, extended program. As hemolytic anemia causes decreased exposure time of Hb to glucose, the result is a decreased percentage of Hb undergoing glycation.⁴ Fructosamine is neither affected by disorders of red blood cells nor influenced by Hb variants;

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Abbreviation: (Hb) hemoglobin, (HbA1c) hemoglobin A1c, (HPLC) high-performance liquid chromatography, (IFCC) International Federation of Clinical Chemistry and Laboratory Medicine

Keywords: fructosamine, hemoglobin A1c, hemoglobin Louisville, hemolytic anemia

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Table 1.
Results of Three Patients with Hb Louisville

Patient	Age (yr)	Sex	FPG (mmol/liter)	HbA1c HA-8160 (mmol/mol (%))	HbA1c Cobas (mmol/mol (%))	Hb Total (g/dl)	Fructosamine (mmol/liter)
A	51	M	11.1 8.9 9.5	7 (2.8) 8 (2.9) 11 (3.2)		10.8 10.5 9.4	----
B	64	F	14.5 12.8 13.0	25 (4.4) 18 (3.8) 7 (3.7)	19 (3.9)	9.7 10.1 10.9	391 420
C	71	F	8.4 7.2 7.5	22 (4.2) 27 (4.6) 17 (3.7)	18 (3.8)	8.8 8.5 8.6	295

FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HA-8160, HPLC Adams A1c HA-8160 of A. Menarini Diagnostic; Cobas, Tina-quant HbA1c Gen 3 in a Cobas 6000.

thus serving as an indicator of blood glucose concentrations for the previous 2 to 3 weeks. Serum fructosamine level determination is advocated in patients with red blood cell disorders or with discrepancies between glucose measurements and HbA1c values.⁵

We therefore determined fructosamine levels with a colorimetric test using nitriblue tetrazolium in alkaline solution, with the following results: patient A (patient deceased); patient B: 420 $\mu\text{mol/liter}$ (in a previous test 391 $\mu\text{mol/dl}$); and patient C: 295 $\mu\text{mol/liter}$ (reference range $>285 \mu\text{mol/liter}$).

Physicians need to be aware of the factors that can influence laboratory HbA1c levels, as therapeutic decisions are often based on these measurements. In diabetes patients who are Hb Louisville carriers, glycemic control should be monitored with serial glucose determinations or by fructosamine level.

Acknowledgments:

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