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Interference of Hemoglobin D on Measurements of Hemoglobin A1c by the High-Performance Liquid Chromatography HA-8160 in 27 Patients

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The accuracy of several hemoglobin A1c (HbA1c) methods can be adversely affected by the presence of hemoglobin (Hb) variants.[1]

In a given period of 6 months, we detected an interference in the measurements of HbA1c in 27 patients living in the island of Gran Canaria (Canary Islands, Spain).

Hemoglobin A1c whole blood measurements were performed using the high-performance liquid chromatography (HPLC) ADAMS A1c HA-8160 method of A.MENARINI Diagnostics in diabetic mode and calibrated in accordance with the International Federation of Clinical Chemistry (IFCC) reference measurement procedure.

In all cases, the chromatogram showed an abnormal pattern that hindered the accurate quantification of HbA1c. In 16 patients, the peaks were not labeled, the chromatogram indicated "abnormal separation," and no result was given for HbA1c. In the remaining 11 patients, the results were abnormally low and not consistent with each patient's respective fasting glucose concentrations.

Therefore, as a comparison, we employed an alternative method, the immunoturbidimetric Tina-quant HbA1c Gen.3 in a COBAS 6000 (Roche Diagnostics), also in accordance with the IFCC standardization. These newer results were consistent with the fasting blood glucose concentrations for each patient (**Table 1**).

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Abbreviations: (DNA) deoxyribonucleic acid, (Hb) hemoglobin, (HbA1c) hemoglobin A1c, (HPLC) high-performance liquid chromatography, (IFCC) International Federation of Clinical Chemistry

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Table 1.
Results of the 27 Patients with Hemoglobin D[a]

Patient number	Age (years)	Sex	FPG (mmol/liter)	HbA1c HA-8160 [mmol/mol (%)]	HbA1c COBAS [mmol/mol (%)]
1[b]	34	Female	4.6	—	30 (4.9)
2[b]	59	Male	5.1	—	30 (5.0)
3[b]	60	Male	5.1	17 (3.7)	31 (5.0)
4[b]	94	Female	5.0	8 (2.9)	33 (5.2)
5	75	Male	5.1	—	38 (5.6)
6	74	Female	5.2	21 (4.1)	40 (5.8)
7	68	Male	7.2	9 (3.0)	41 (5.9)
8	43	Female	6.3	—	42 (6.0)
9	71	Female	6.9	22 (4.2)	43 (6.1)
10	86	Female	5.8	23 (4.3)	43 (6.1)
11	88	Female	8.9	—	49 (6.6)
12	79	Female	7.1	29 (4.8)	50 (6.7)
13	68	Male	5.7	—	51 (6.8)
14	58	Female	7.5	—	51 (6.8)
15	75	Male	10.1	—	52 (6.9)
16	67	Male	9.9	—	54 (7.1)
17	76	Female	5.5	—	56 (7.3)
18	60	Male	7.6	31 (5.0)	57 (7.4)
19	76	Female	10.1	—	67 (8.3)
20	68	Female	10.7	40 (5.8)	68 (8.4)
21	70	Male	10.9	—	73 (8.8)
22	80	Male	9.6	—	74 (8.9)
23	52	Male	11.5	—	78 (9.3)
24	66	Female	14.6	—	85 (9.9)
25	59	Female	4.9	—	88 (10.2)
26	59	Female	4.9	—	88 (10.2)
27	61	Male	16.8	49 (6.6)	92 (10.6)

[a] FPG, fasting plasma glucose; HA-8160, HPLC ADAMS A1c HA-8160 of A.MENARINI Diagnostic; COBAS, Tina-quant HbA1c Gen.3 in a COBAS 6000 (Roche Diagnostics).
[b] Nondiabetic patients.

Blood samples from the 27 patients were sent to a hemoglobinopathy referral center in Madrid, Spain, and that particular extra peak was labeled as Hb D by cation-exchange HPLC (Variant Bio-Rad). Molecular characterization was performed by deoxyribonucleic acid (DNA) genomic extraction (BioRobot EZ1; Qiagen, Hilden, Germany) and selective amplification of the β gene in an Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster City, CA). The β gene was sequenced with the ABI Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA) and was analyzed in an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems). The DNA sequence of the patient's Hb β gene revealed Hb D Punjab, also called Hb D Los Angeles. This structural Hb variant contains a substitution of glutamine for glutamic acid at position 121 of the β-globin chain. It is usually found in the Sikhs of the Punjab region of the Indian subcontinent. Within our group of 27 patients, 5 were of Asian descent.

The presence of lower HbA1c by HPLC in patients carrying the Hb D trait has been reported previously.[2–4] In our current study, we found no results in 59% of patients and abnormal results in the remaining 41%.

We conclude that, in diabetes patients who were heterozygous for the Hb D variant, determining HbA1c levels using the ADAMS HA-8160 HPLC method gave falsely low or unquantifiable results due to an abnormal separation of HbA1c. We therefore recommend that all chromatograms be thoroughly reviewed prior to the release of a HbA1c result to identify possible interference from Hb variants, particularly when the result is inconsistent with the patient's fasting blood glucose concentrations or medical history. Additionally, in Hb D variant carriers, we recommend measurement of HbA1c using other alternatives, such as a turbidimetric immunoassay Tina-quant HbA1c Gen.3, which is less likely to be subject to interference when the mutations are present much farther from the N-terminus of the Hb β chain.

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