Continuing Diagnostic Relevance of the Sickling Test in the Era of CE-HPLC

Meet Kumar · Prashant Sharma · Vijay Kumar ·
Manorama Bhargava

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Dear Editor,

Cation-exchange high performance liquid chromatography (CE-HPLC) has become the most common method used to screen for hemoglobin variants. However, identification is only provisional and current guidelines emphasize that all chromatographically detected abnormal hemoglobins must subsequently be verified by an independent second technique [1]. Sick cell hemoglobin (HbS)-related disorders are common in India [2]. CE-HPLC is highly specific for HbS in the Indian setting since the S-window co-eluting variants (retention time 4.3–4.7 min) like HbQ-Thailand, HbG-San Jose, and Hb-Manitoba are few and moreover, are extremely rare in Indians [2–4]. We recently encountered two related patients with a non-sickling hemoglobin eluting in the S-window where a routine time-tested sickling test with sodium metabisulfite–sodium dithionite prevented a misdiagnosis.

A Sudanese mother and daughter, who were in India on a business visit, underwent a routine health check-up that revealed very mild hypochromic anemia (Table 1). Their serum iron parameters were within normal limits. CE-HPLC (BioRad variant II system, β -thal short programme) revealed peaks in the HbS-window in both mother (32.9%) and daughter (32.7%) with an identical retention time of 4.61 min (Fig. 1). Although, a diagnosis of sickle cell trait

was entirely consistent with their African ancestry, sickling tests with sodium metabisulfite–sodium dithionite were done as is routine in our laboratory for all HbS-window peaks. To our surprise, no sickling occurred at 24 h in either patient. Repeat testing using freshly constituted reagents was also consistently negative.

Since the second-line modality had failed to confirm HbS, Hb electrophoreses at pH 8.4 was done (SAS-II, Helena BioSciences, Europe). This showed a prominent band for HbA along with a slow-moving band corresponding to the region of Hb S/D/G/Q (Fig. 2). The differential diagnoses at this stage therefore were HbG-San Jose (RT 4.61) and HbQ-Thailand (RT 4.67). Hb-Manitoba was excluded as it migrates just anodal to HbA at alkaline pH [5–7].

To differentiate Q-Thailand from G-San Jose, Hb electrophoresis at pH 6.4 was performed. This revealed a band for HbA and another band that merged with it but was faster than HbC and HbS (Fig. 3). This definitively excluded HbS and HbG-San Jose (as G-San Jose migrates between HbC and HbS at acid pH [8]), thereby leading to a presumptive identification of the variant as HbQ-Thailand [9]. Genetic testing was offered, however, the patients declined on being counseled that the variant was unlikely to be of clinical significance to the probands.

HbQ-Thailand occurs due to the mutation $\alpha 74$ (EF3) Asp > His in the $\alpha 1$ -globin gene. Its co-inheritance with $\alpha 2$ -globin gene deletion is well recognized to yield a high abnormal variant of 30–35% with thalassemic red cell indices as in our cases [9].

In conclusion, our finding of this unusual variant hemoglobin makes a strong argument in favor of the routine confirmation of all S-window CE-HPLC peaks by the simple, cost-effective and rapid test for sickling to avoid misdiagnoses. In addition, it is a reminder to hematopathologists

M. Kumar · P. Sharma (✉) · V. Kumar · M. Bhargava
Department of Haematology, Sir Ganga Ram Hospital,
1st Floor-SSRB, Rajinder Nagar 110060, New Delhi, India
e-mail: Prashant.Sh@gmail.com

Table 1 Blood count and CE-HPLC parameters in the two patients

Complete blood counts			
Parameter (unit)	Mother's results	Daughter's results	Normal range
Hemoglobin (g/dl)	11.7	11.4	12–16
RBC count (million/ μ l)	5.10	5.00	4.20–5.40
PCV (%)	36.5	34.8	37.0–47.0
MCV (fl)	71.6	69.6	82.0–97.0
MCH (pg)	20.1	22.7	28.0–32.0
MCHC (g/dl)	32.0	32.7	32.0–36.0
TLC (/ μ l)	9,400	6,700	4,000–11,000
Platelets (/ μ l)	3,59,000	2,54,000	1,50,000–3,00,000
RDW (%)	21.7	19.4	11.5–14.5
Hemoglobin CE-HPLC fractions			
HbF (%)	0.1	0.3	<1.5
HbA (%)	56.6	57.0	–
HbA2 (%)	2.8	2.7	1.9–3.5
S-window (%)	32.9	32.7	–

Fig. 1 CE-HPLC of the mother showing an abnormal hemoglobin peak with a retention time of 4.61 min in the S-window. HbA and HbA2 were also present. The chromatogram of the daughter was very similar **Fig. 2** High resolution image of the Hb electrophoresis at alkaline pH. The patients' samples are in lanes 1 and 2. The other lanes (diagnoses indicated) as well as the adult and fetal controls (lanes 11 and 12) serve as references. The abnormal Hb of the index cases migrates to the region of Hb S/D/G/Q **Fig. 3** High resolution image of the Hb electrophoresis at acid pH. The patients' samples are in lanes 1 and 2. The other lanes (diagnoses indicated) as well as the adult and fetal controls (lanes 11 and 12) serve as references. The index cases show bands for HbA merged with a slower moving band in the region of HbQ. This corresponds to the HbQ-India cases in lanes 8 and 9, but not the HbS cases in lanes 4 and 6

that in this era of medical tourism, one needs to consider rare Hb variants in their differential diagnoses irrespective of ethnic boundaries.

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