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CORRESPONDENCE

Unusual C- and A2-Window Peaks (Hemoglobin M-Saskatoon) in Three North Indian Patients

Ojas Gupta[1] · Sanjeev Chhabra[1] · Jasbir Kaur[1] · Reena Das[1] · Deepak Bansal[2] · Pankaj Malhotra[3] · Prashant Sharma[1]

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Dear Sir,

We read with interest the report by Dass et al. [1] wherein they concluded that CE-HPLC-derived C-window peaks in north Indian patients only rarely represent HbC. In their findings, all four non-HbC C-window peaks were due to a hybrid HbQ^{India} + HbD^{Punjab}, a finding also previously reported by us [2]. We would now like to add three cases of HbM-Saskatoon, an unusual methemoglobinemic variant, to the differential diagnosis of variant hemoglobins that result in C-window peaks.

Our first two cases were a father-son duo. The 1.5-year-old boy (**Case 1**) born from a non-consanguineous marriage was reportedly healthy at birth with a normal birth weight and no obvious congenital anomalies. He was referred to our center for febrile seizures. On examination, central cyanosis, mild pallor and jaundice were noted. Liver was just palpable while the spleen was non-palpable. Cardiovascular and respiratory system evaluations were normal. Hemogram data is presented in Table 1. Blood film showed mild anisopoikilocytosis with normocytic normochromic to macrocytic red cells.

Further investigations revealed methemoglobinemia (7.5%, method of Evelyn and Malloy) [3] with a normal glucose-6-phosphate-dehydrogenase screen. Cation-exchange high-performance liquid chromatography (CE-HPLC, Fig. 1a) using Variant II™ analyser (BioRad Laboratories, Hercules, USA) revealed 1.7% HbF, 20.6% HBA_2 + variant, an unknown peak (2.5%, retention time 4.82 min) plus a C-window peak (7.2%, 5.05 min). Hemoglobin electrophoresis at pH 8.6 was normal. Heat stability and isopropanol tests for unstable hemoglobins were positive. Spectral analysis of hemolysate at 400 and 700 nm showed shifted peaks at 602 nm and 492 nm from normal absorption maxima at 632 nm and 502 nm respectively, consistent with HbM-pattern. *HBB* gene sequencing done on an ABI Prism 3100 Genetic analyzer (ThermoFisher Scientific, USA) showed a CAT → TAT change (histidine → tyrosine) in heterozygous state at codon 63 of the β-globin gene, confirming HbM-Saskatoon [β63(E7)His → Tyr].

His father aged 26 years (**Case 2**) also had a history of chronic cyanosis, jaundice in childhood and one prior blood transfusion at the age of 13 years. He had not been investigated previously and was currently in good health. Hemoglobin HPLC and spectral analysis showed similar findings as his son (Table 1). The child's mother and paternal grandmother were clinically and hematologically normal.

Case 3 was an unrelated 40-year-old male with cyanosis and mild jaundice referred for Hb-HPLC without any further clinical data. HPLC findings showed similar findings as that of case 1 (Table 1, Fig. 1b). Sanger sequencing confirmed heterozygosity for the β63(E7)His → Tyr variant in this patient as well (Fig. 1c).

Methemoglobins are characterized by the persistent oxidation of heme–iron from ferrous to ferric state, where

✉ Prashant Sharma
sharma.prashant@pgimer.edu.in

[1] Department of Hematology, Level 5, Research Block A, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India

[2] Pediatric Hemato-Oncology Unit, Advanced Pediatric Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, India

[3] Adult Clinical Hematology Unit, Department of Internal Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Table 1 Laboratory findings in the three cases

	Case-1	Case-2	Case-3
Hemoglobin (g/L)	95	117	121
Hematocrit (%)	30.1	37.5	37.9
Red blood cell count (/l)	3.34×10^{12}	3.57×10^{12}	3.8×10^{12}
Mean cell volume (fl)	90.3	104.9	98.7
Mean cell hemoglobin (pg)	28.4	32.7	31.5
Mean cell hemoglobin concentration (g%)	31.5	31.2	31.9
Red cell distribution width—CV (%)	17.3	14.1	13.9
Total leucocyte count ($\times 10^9$/l)	12.1	4.7	7.0
Platelet count ($\times 10^9$/l)	333	178	174
Reticulocyte count (%)	2.85	3.39	NA
Methemoglobin	7.5% (after blood transfusion—0.3%)	5.0%	NA
Hb CE-HPLC results			
HbF	1.7%	0.4%	0.2%
HbA0	61.6%	62.6%	65%
HbA2 + variant	20.6%	21.3%	15.1%
Unknown peak% (retention time in minutes)	2.5% (4.8)	2.4% (4.8)	6.3% (4.8)
C-Window peak% (retention time in minutes)	7.2% (5.0)	7.1% (5.1)	7.7% (5.1)

NA not available

the ferric form (Fe^{3+}) is more stable than the usual ferrous (Fe^{2+}). They can occur due to globin gene mutations (hemoglobin M) causing amino acid substitution in the heme pockets of the encoded globin chain that directly affects the hemoglobin bond [4]. Alternatively, they may occur due to deficiency of cytochrome b5-reductase enzyme.

Congenital Hb Ms include α-chain variants like M-Iwate, β-chain variants like M-Hyde Park and ϒ-chain variants like HbF M-Osaka. Patients usually present with cyanosis or pseudocyanosis. Treatment is not indicated however accurate diagnosis is important for reassurance and to prevent unnecessary investigations [3, 4]. HbM-Saskatoon was first reported in Japan, followed by reports from USA, Indonesia, Algeria, Russia and Germany. A prior Indian report is by Kedar et al. in 2005 as HbM-Ratnagiri [5].

In conclusion, our series of three cases of HbM-Saskatoon from two families highlights this rare cause of congenital cyanosis, and reminds hematopathologists of a rare entity that presents with highly unusual Hb HPLC chromatograms with C-window peaks.

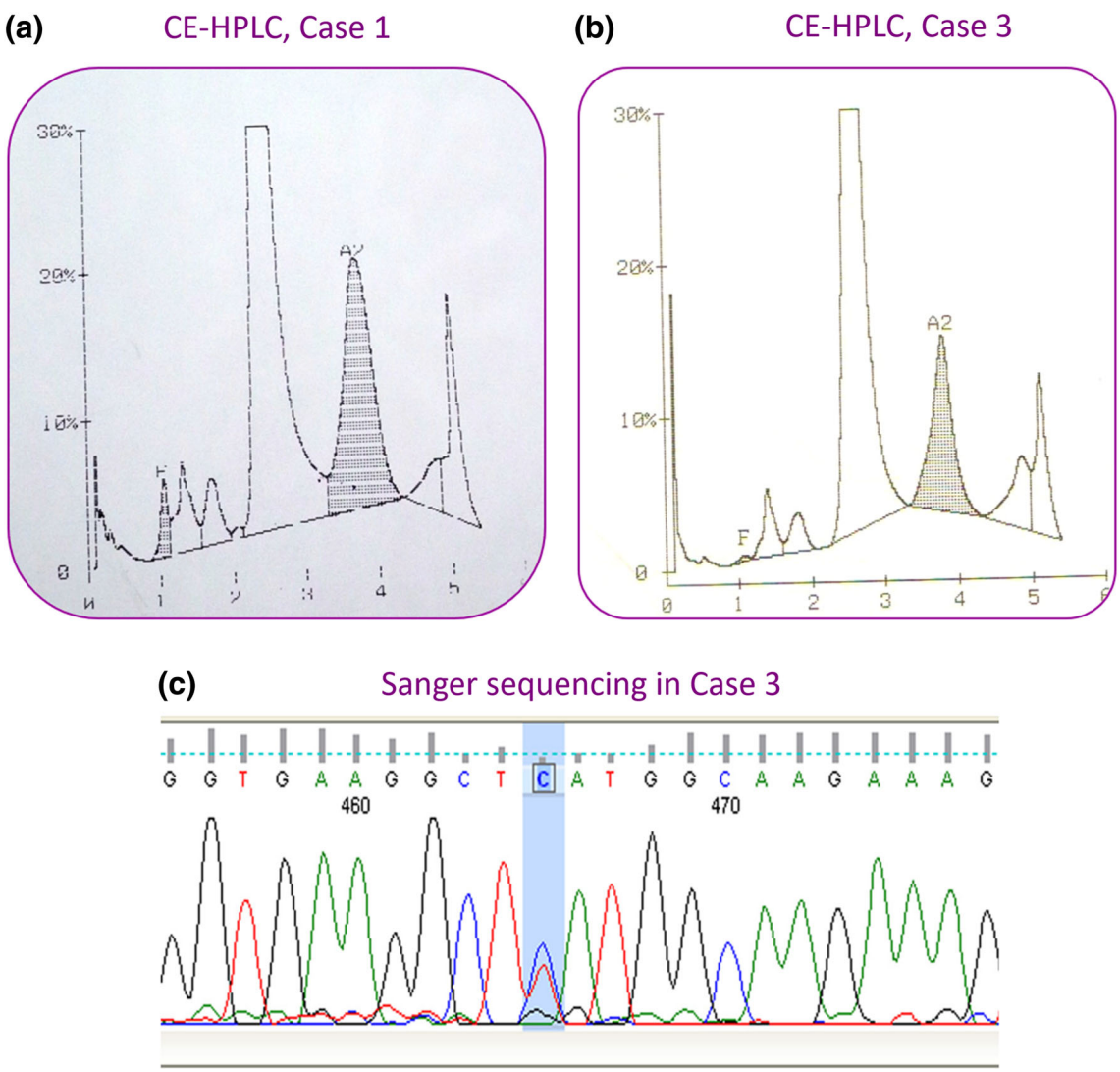

Fig. 1 **a**, **b** HPLC chromatograms from patients 1 and 3 respectively. **c** Sanger sequencing chromatogram from case 3 shows a heterozygous C > T substitution

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethical Approval All procedures performed in this report involving a human participant were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This is a purely observational report and no research procedure was done.

Informed Consent Informed consent has been obtained for this manuscript.

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