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CORRESPONDENCE

Hemoglobin Titusville [α2 Codon 94 G>A]: A Rare Alpha Globin Chain Variant Causing Low Oxygen Saturation

Amar Das Gupta[1] · Priya Hariharan[2] · Manisha Daruwalla[1] · Kainaz Sidhwa[1] · Ravikiran Pawar[1] · Anita Nadkarni[2]

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Dear Editor,

Alpha globin chain variants are usually not associated with the morbidity and mortality that are observed in beta globin chain hemoglobinopathies. Hemoglobin (Hb) Titusville (HBA2:c.283G>A), however, is a rare alpha globin gene defect (alpha2 globin gene codon 94 GAC>AAC mutation) wherein the resultant amino acid substitution at the α1β2 globin chain contact leads to reduced oxygen affinity of the Hb molecule [1]. We report here a 31 years old woman from Tiruchirappalli, Tamil Nadu in whom Hb Titusville resulted in low oxygen saturation during surgery.

The patient's oxygen saturation was found to be 80–84% on pulse oximetry (with normal arterial blood gas levels) during spinal anesthesia for a caesarean section (CS). The CS was completed with oxygen support and the patient had an uneventful child birth. The baby did not show cyanosis at her birth. She could not be investigated further. However, we could investigate the patient's mother.

The patient and her mother showed normal Hb and red cell indices (Table 1). High performance liquid chromatography (HPLC) on Variant II HPLC system (Bio Rad, Hercules, USA) revealed small abnormal hemoglobin peaks—13.8% and 13% in the patient and her mother respectively—eluting in the HbS window with a retention time of 4.53 min (Fig. 1a). HbA_2 was 1.9 and 2.1% respectively. On cellulose acetate (CA) electrophoresis (pH 8.9) the abnormal Hb had the same electrophoretic mobility as HbS (Fig. 1b). Heat stability test was negative. These findings and a negative sickling test prompted sequencing of the patient's DNA on 3130 XL genetic analyser (Applied Biosystems, Foster City, USA) that confirmed heterozygous state for Hb Titusville resulting from an alpha2-globin gene codon 94 GAC→AAC mutation (Fig. 1c). Gene sequencing was not performed in the patient's mother.

The molecular lesion in Hb Titusville results in replacement of aspartic acid in position 94 in the alpha globin chain by asparagine [1]. Aspartic acid at position 94 normally interacts with asparagine on position 102 which occurs at the α1β2 interface through strong hydrogen bonding in the quaternary oxyhemoglobin structure. This change therefore, impairs oxygen binding ability of the variant hemoglobin molecule resulting in its low oxygen affinity and shifting of the oxygen dissociation curve to the right [2, 3]. Other variant hemoglobins having amino acid substitution at the same position [Hb Bassett (alanine), Hb Capa (glycine), Hb Roanne (glutamic acid), Hb Setif (tyrosine) and Hb Sunshine Seth (histidine)] also characteristically show a low oxygen affinity [3]. Though low O_2 affinity Hb variants have enhanced tissue oxygenation property, they can also be associated with mild anemia and cyanosis due to abnormally high levels of deoxy Hb [4].

This report emphasises the role of a stepwise diagnostic approach for detecting hemoglobin variant that might otherwise be misclassified as HbS. Low HbS levels in sickle cell heterozygotes, not uncommonly observed in our practice, may be due to co-inheritance of alpha thalassemia or rarely, a beta thalassemia mutation in cis, or concomitant iron deficiency, or a recent blood transfusion to treat anemia due to an unrelated cause. However, the very findings of low level of the abnormal hemoglobin, and

✉ Amar Das Gupta
adasgupta@srl.in

[1] Sections of Hematology, Coagulation and Flow Cytometry, SRL Diagnostics, Mumbai, India

[2] Division of Hematogenetics, National Institute of Immunohematology, Mumbai, India

Table 1 Red cell parameters and Hb fractions separated on HPLC in the patient and her mother

Parameters	Patient	Patient's mother
RBC ($\times 10^9$/l)	3.91	4.52
Hemoglobin (g/dl)	11.6	12.8
Hematocrit (%)	36.0	40.9
Mean corpuscular volume (fl)	92.1	90.6
Mean corpuscular hemoglobin (MCH) (pg)	29.6	28.3
MCH concentration (g/dl)	32.2	31.2
Red cell distribution width (%)	15.8	24.2
HbA (%)	76.1	74.4
HbA2 (%)	1.9	2.1
HbF (%)	0.2	0.2
HbX (%) (retention time 4.53 min)	13.8	13.0

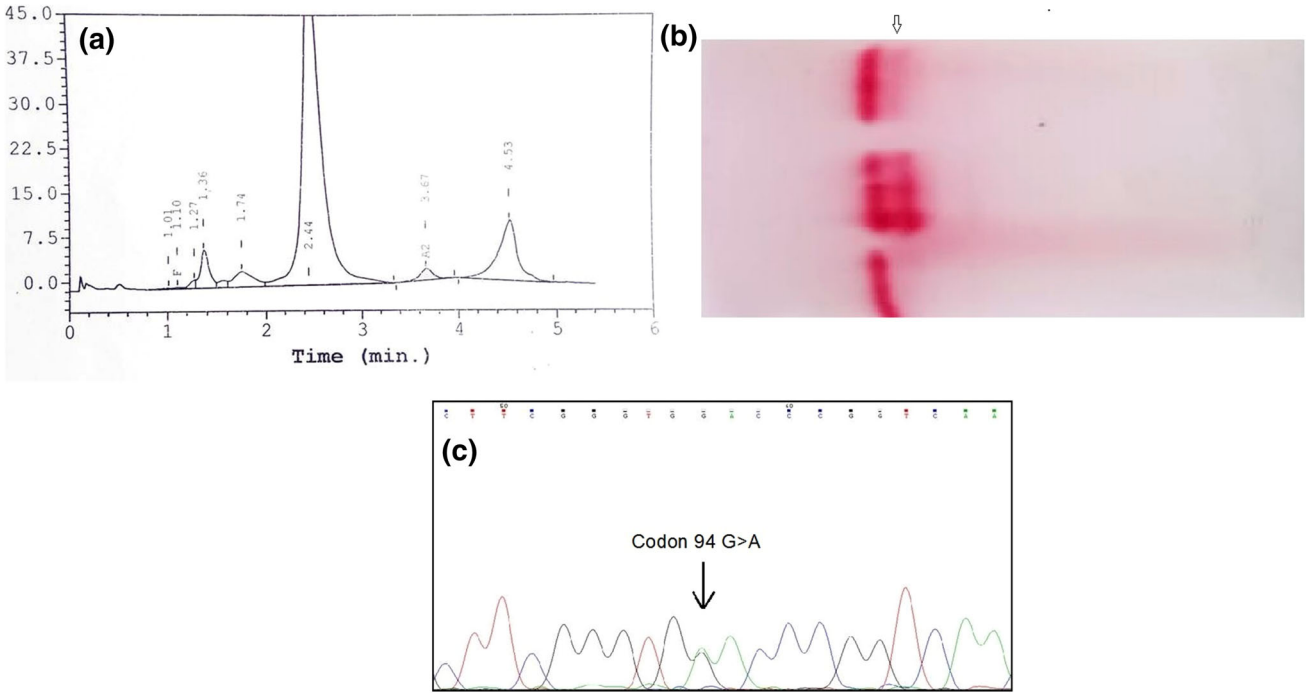

Fig. 1 **a** HPLC histogram of the patient showing the small abnormal Hb peak (13.8%) of Hb Titusville in HbS window (retention time 4.53 min); **b** Hb Titusville band (arrow) of the patient in cellulose acetate electrophoresis; **c** gene sequencing showing codon 94 GAC>AAC mutation in alpha 2 globin gene in the patient

negative sickling test in this case suggested the possibility of an alpha globin chain variant rather than HbS. As Hb Titusville shows migration properties similar to HbS in CA electrophoresis at alkaline pH (Fig. 1b), this test was not helpful in differentiating between the two. The presence of Hb Titusville in heterozygous state was finally confirmed by gene sequencing studies.

Two cases of Hb Titusville (father and his newborn daughter) were recently reported in an Indian family residing in Australia [5]. So, to the best of our knowledge, the cases being reported by us are the first such cases from India. Although the clinical and hematological features in the Australian cases were similar to ours, the baby in the former instance showed the presence of HbF Titusville in view of high HbF level and HbA2 Titusville in capillary electrophoresis. These abnormal Hbs had resulted from combination of abnormal alpha globin chain of Hb Titusville with normal gamma and delta globin chains respectively. We did not observe any HbA2 Titusville in the patient or her mother on HPLC.

Compliance with Ethical Standards

Conflict of interest The Authors declare that they have no conflict of interest.

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