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CASE REPORT

Hemoglobin Reims—a rare alpha globin chain variant and its interaction with beta thalassemia

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Abstract

Non-sickle hemoglobin (Hb) variants that elute in HbS window in high performance liquid chromatography (HPLC) pose diagnostic challenges, especially in HbS prevalent geographies. We describe here two brothers (patients 1 and 2) with Hb Reims, a rare alpha globin chain variant that eluted in HbS window. Hb analysis was performed by HPLC. Covalent reverse dot blot and refractory mutation system (ARMS) were used for detection of common beta globin gene mutations. Alpha and beta globin gene mutation analysis was performed by DNA sequencing. Both brothers had "thalassemia trait-like" red cell indices. HbA_2 was high (4.9%) in patient 2 and normal (2.7%) in patient 1. HbF was normal (0.3%) in both. The abnormal Hb peaks in patient 1 (21.7%) and patient 2 (13.8%) eluted at 4.51 and 4.48 min, respectively, in HPLC. Sickling test was negative in both. Gene sequencing confirmed heterozygous Hb Reims in both brothers resulting from an HBA1:c.71AC, GluGly; Codon 23 (GAG→GGG) mutation of alpha 1 globin gene. Both also had an alpha globin gene deletion ($-\alpha^{3.7}/\alpha\alpha$). Patient 2 additionally had heterozygous beta thalassemia resulting from Codon 15 (G→A) beta globin gene mutation. Hb Reims is a clinically silent Hb variant that needs to be distinguished from HbS. A co-existent beta thalassemia seems to have lowered the level of Hb Reims in patient 2. Only one case of Hb Reims has been reported earlier in the world literature and none from India where the two brothers hail from.

Keywords Hb Reims · Alpha globin chain variants · Non-HbS in HbS window · Interaction between beta thalassemia and alpha chain variants

Introduction

High-performance liquid chromatography (HPLC) is widely used for screening of hemoglobin (Hb) abnormalities. Hb variants are identified in HPLC on the basis of their retention time with respect to the predefined elution windows and the contour of the Hb peak [1]. A number of non-sickle Hb (non-HbS) variants elute in the HbS window causing diagnostic dilemma, especially in communities that have a high incidence of HbS. Most of these variants are not associated with a disease state. Hence, it is important to identify and distinguish the non-pathogenic variants from the pathogenic ones (e.g., HbS). Here, we describe two brothers with Hb Reims, a rare alpha globin chain variant that elutes in HbS window in HPLC [2]. One of the brothers had also co-inherited a beta thalassemia trait, thereby allowing us to look at the influence of beta thalassemia trait on the level of the variant Hb under discussion. To the best of our knowledge, this is the first report of Hb Reims from India.

Case history

Two brothers—patient 1 (44 years) and patient 2 (39 years)—were referred to our laboratory for hemoglobinopathy screening since the father was diagnosed to have "thalassemia." They were born of non-consanguineous marriage and both were in good health. There was no past history of low Hb, jaundice, cyanosis, bone or joint pain, and heaviness or pain in the abdomen. There was no organomegaly. Neither of the bothers received blood transfusion in the past. The parents' blood samples could not be obtained

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in spite of all efforts. The family had migrated to greater Mumbai area from the state of Uttar Pradesh in north India a couple of generations back.

Materials and methods

Standard techniques were used for complete blood count and sickling tests [3]. Hb analysis was performed on Variant II HPLC system (Bio Rad, Hercules, USA). DNA was extracted using the Flexigene Qiagen Kit. Molecular analysis of common beta globin gene mutation was performed by covalent reverse dot blot and ARMS refractory mutation system method [4]. Whole beta globin gene mutation analysis was performed by direct sequencing of the patient's DNA on 3730 XL genetic analyzer (Applied Biosystems, Foster City, USA) [5]. Multiplex PCR was used to detect common alpha gene deletions.

Informed consent was obtained from the patients for all investigations and for publishing the details.

Results

Both brothers (elder brother referred to here as patient 1; younger brother as patient 2) had normal Hb but had "thalassemic" red cell indices, i.e., high red cell count, low mean corpuscular volume (MCV), low mean Hb content (MCH), normal mean corpuscular Hb concentration (MCHC), and normal or near normal red cell distribution width (RDW) (Table 1). Correspondingly, the red cells appeared microcytic in the blood smear.

HPLC showed an abnormal Hb peak (HbX1) in HbS window in both patients—patient 1 (21.7%) and patient 2 (13.8%), with retention times (RT) of 4.51 and 4.48 min, respectively (Fig. 1 and Table 1). Sickling was negative and HbF was normal (0.3%) in both. HbA2 was normal (2.7%) in patient 1 while it was elevated (4.9%) in patient 2. Both brothers also showed a minor Hb peak (HbX2) (0.6% and 0.9%, respectively) at RT of 4.69 min, immediately after the main abnormal Hb peak. The HPLC results are summarized in Table 1 and explained in Fig. 1a and b.

Globin gene sequencing in both brothers revealed heterozygous state of a novel alpha globin gene mutation—Hb Reims [HBA1:c.71A→G, Glu→Gly; Codon 23 (GAG→GGG)] along with single α1 globin gene deletion ($-\alpha^{3.7}/\alpha\alpha$) *Typeequationhere*.as detected by multiplex PCR (Figs. 2 and 3). Patient 2 also showed the presence of heterozygous beta thalassemia [β globin gene mutation–Codon 15(G→A)] in addition to Hb Reims (Figs. 4 and 5), pointing to a double heterozygous state for Hb Reims and beta thalassemia.

Table 1 Red blood cell (RBC), blood smear, sickling, and HPLC findings in the two brothers (patients 1 and 2). Note the high red cell counts and the "thalassemic" red cell indices in both the brothers. Also note the lower level of the abnormal Hb (HbX1 or Hb Reims) in patient 2 along with a high HbA2 suggesting coinheritance of beta thalassemia

RBC and HPLC findings in patients		
Parameters	Patient 1	Patient 2
Hb (g/dl)	15.4	14.8
RBC ($\times 10^6$/μl)	6.12	6.48
MCV (fl)	77.3	71.9
MCH (pg)	25.2	22.8
MCHC (%)	32.6	31.8
RDW (% cv)	15.4	16.8
RBC morphology	Mildly microcytic hypochromic RBCs; target cells +	Mildly microcytic hypochromic RBCs; target cells +
HbA	66.4	69.8
HbF	0.3	0.3
HbA2	2.7	4.9
HbX1	21.7% (RT = 4.51 min)	13.8% (RT = 4.48 min)
HbX2	0.6% (RT = 4.69 min)	0.9% (RT = 4.69 min)
Sickling	Negative	Negative

Discussion

With increasing use of HPLC in primary screening of hemoglobinopathies, a number of non-HbS variants that elute in Hb S window have been identified. Some of these have been reported from the Indian subcontinent [6–10]. Therefore, our case assumes a greater clinical significance. The absence of sickling in sodium metabisulfite preparation [3] in these cases is an initial pointer towards their being non-HbS variants. The awareness of these facts is necessary for planning further laboratory workup for correct characterization and identification of the variant Hb under these circumstances. It is also important to realize that some of these non-HbS variants, e.g., Hb Titusville [8], cause morbidity in the patients by lowering oxygen (O_2) affinity and O_2 saturation of blood, and they need to be differentiated from HbS upfront.

Hb Reims is a rare alpha globin chain variant [alpha 23 GAG→GGG (Glu→Gly)] that elutes in HbS window (4.30–4.70 min) in HPLC. In patients 1 and 2, Hb Reims eluted at RT of 4.51 and 4.48 min, respectively. This Hb variant was first reported in 1989 in a 60-year-old French-Caucasian woman [2]. It is interesting to note that no other case of Hb Reims has been reported in the world literature since then. So, it appears that ours are the only second and third cases of Hb Reims per the literature search conducted by us. These are also the first two cases of Hb Reims reported from India.

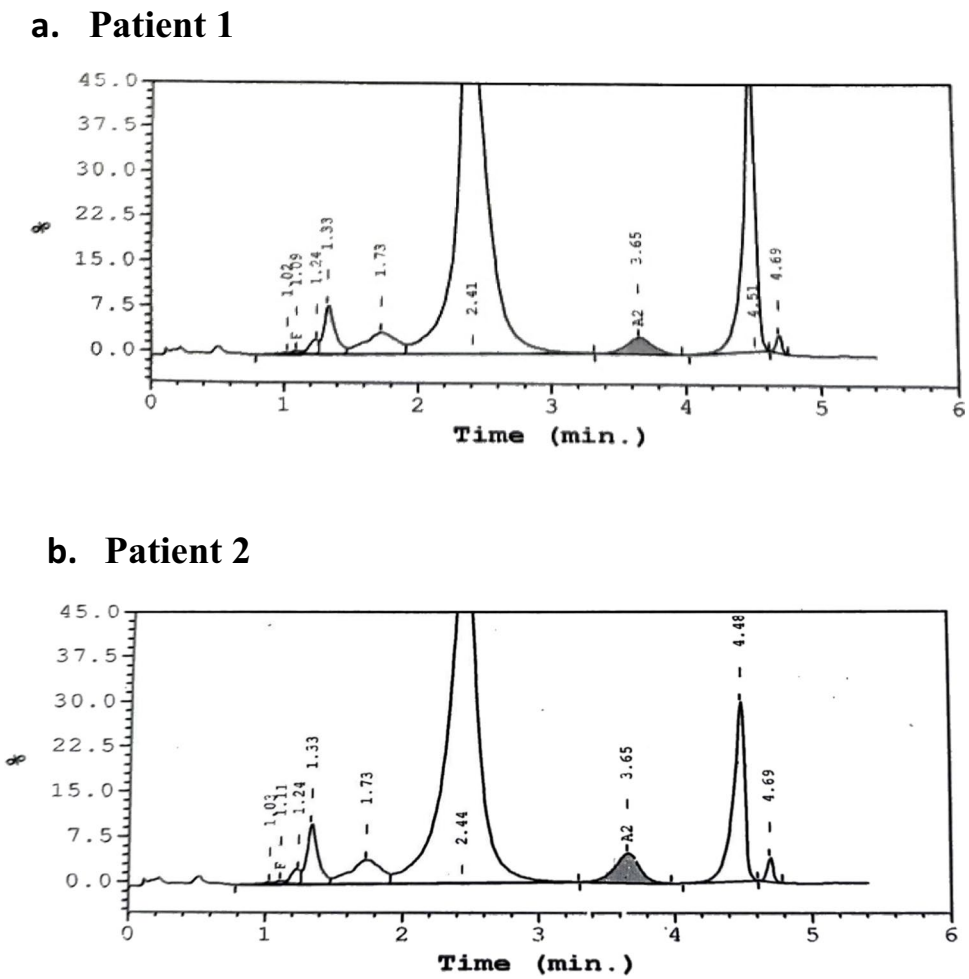

Fig. 1 HPLC elution patterns seen in patient 1 (**a**) and patient 2 (**b**). Note the abnormal Hb peaks representing Hb Reims with retention time (RT) of 4.51 min in patient 1 and 4.48 min in patient 2. Also note the high HbA2 in patient 2. An additional small abnormal Hb peak with RT of 4.69 min, possibly representing "HbA2 Reims," is seen in both the brothers

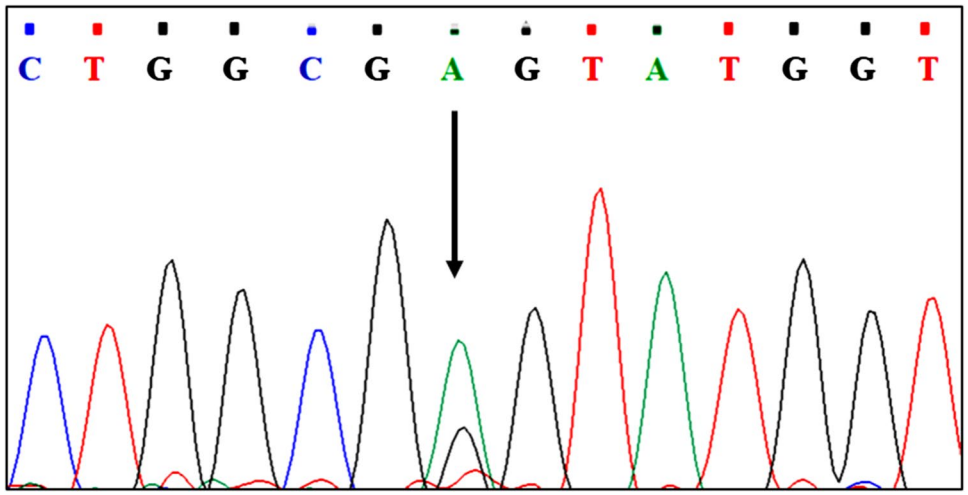

Fig. 2 Alpha globin gene sequencing in patient 1 showing A > G substitution in codon 23 in Hb Reims that resulted in substitution of Glutamic acid by Glycine in alpha globin peptide chain

Unlike some other variants resulting from mutation at Codon 23 in alpha globin gene, e.g., Hb Memphis (Glu→Gln) [11], Hb Chad (Glu→Lys) [12] and Hb G-Audhali (Glu→Val) [13] Hb Reims is not associated with any change in oxygen affinity [2]. However, the index case reported earlier [2] showed mild instability of Hb Reims. This was not tested in our cases.

As in the previously reported case of Hb Reims [2], our patients too were asymptomatic and had normal Hb but showed 'thalassemic' red cell indices, i.e. high red cell count, low MCV, low MCH and normal RDW. Both also had normal Hb as in the case published earlier [2]. Molecular analysis revealed an associated single alpha1

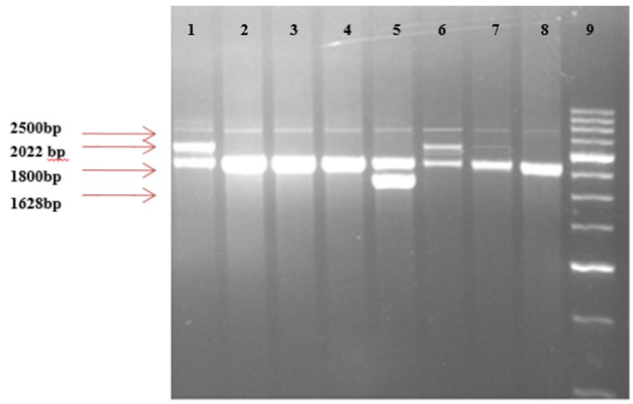

Fig. 3 Detection of α globin gene deletion by multiplex PCR. Lanes 1 and 6 show alpha gene deletion ($-\alpha^{3.7}/\alpha\alpha$) in patients 1 and 2, respectively. Lane 7: positive control. Lanes 2, 3, 4, and 8 are normal controls (αα/αα). Lane 5 shows $-\alpha^{4.2}/\alpha\alpha$ deletional control and lane 9 DNA marker XVI

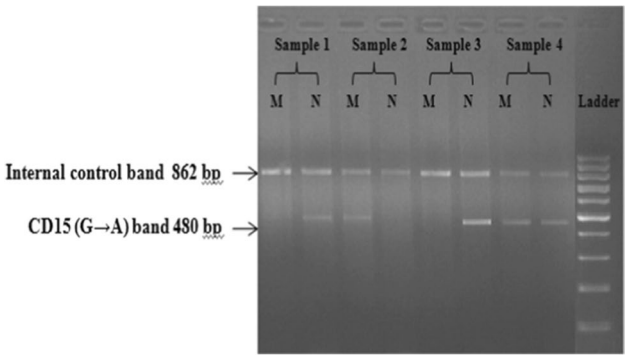

Fig. 5 Detection of β globin gene mutation by ARMS-PCR in 2% agarose gel. *M* mutant, *N* normal. Normal control (sample 1; lanes 1 and 2): absence of CD 15 (G→A) mutation (only normal band is seen in lane 2). Homozygous for CD 15 (G→A) mutation (sample 2; lanes 3 and 4): only 480 bp mutant band is seen in lane 3. Patient 1 (sample 3; lanes 5 and 6): absence of CD 15 (G→A) mutation (only normal band is present in lane 6). Patient 2 (sample 4; lanes 7 and 8): heterozygous for CD 15 (G→A) mutation (both 480 bp mutant and normal bands are present). Lane 9: 100-bp DNA ladder

globin gene deletion ($-\alpha^{3.7}/\alpha\alpha$) leading to deletional alpha thalassemia and the red cell changes observed in our cases are in line with this diagnosis. Bardakd jian-Michau et al. [2] too suspected an alpha thalassemia 2 type defect in their patient on the basis of the limited analysis performed by them. Genetic analysis in our cases further showed that patient 2 additionally had a beta thalassemia trait due to beta globin gene mutation at codon 15(G→A) (Fig. 3).

It is noteworthy that Hb Reims level was significantly lower (13.8%) in patient 2 who also had co-inherited beta thalassemia trait as compared with patient 1 (21.7%). The possibility of the coexisting beta thalassemia lowering the level of Hb Reims (an alpha chain variant) in patient 2 is strong as observed in double heterozygotes for beta thalassemia and some other alpha chain variants such as HbQ India [14] and Hb Rampa [15]. This indicates a preferential formation of HbA over that of alpha chain variant Hb in conditions of relative beta globin chain deficiency such as beta thalassemia. These observations also suggest that the rate of dimer or tetramer formation from monomers can be an important mechanism of controlling the quantity of certain hemoglobin variants with critical substitutions in heterozygote state [15].

The additional small abnormal Hb peaks (HbX2, Table 1) with retention time of 4.69 min, immediately after the Hb Reims peak in HPLC in our patients, could represent a variant HbA2 resulting from combination of abnormal alpha globin chain of Hb Reims with normal delta chains. Bardakdjian-Michau et al. too had observed the presence of abnormal HbA2 in their case [2]. The higher value of the variant

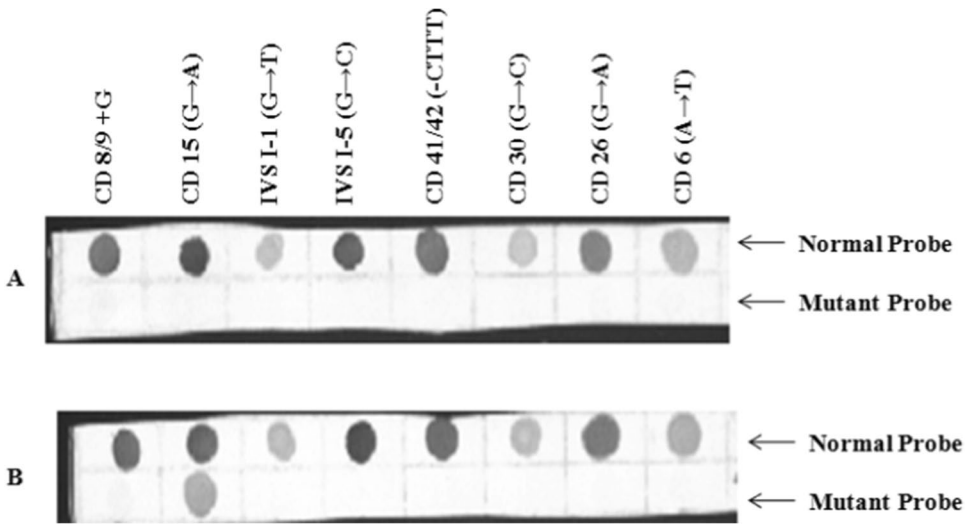

Fig. 4 Detection of common thalassemic β globin gene mutation CD15(G→A) in patient 2 (row B) by covalet reverse dot blot technique (CRDB). Patient 1 (row A) does not show this mutation

HbA2 in patient 2 (0.9% vs 0.6%), who also has a higher HbA2 compared to patient 1 (4.9 vs 2.7), points to the aforementioned mechanism of production of this minor Hb fraction. Similar small abnormal Hb peaks have been observed in HPLC chromatograms of other alpha chain variants such as HbQ India [14].

The two cases reported by us here highlight the need for awareness of uncommon Hb variants that elute in HPLC windows in which more common variants such as HbS and HbE elute, and the role of a systematic, step-wise approach in the diagnosis of the former variants. This will avoid misdiagnosis of these variants.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Amar Dasgupta, Millu Jain, Manju Goriwale, Anita Nadkarni, and Trupti Shetty. The first draft of the manuscript was written by Amar Dasgupta, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Ethical approval The study was approved by the institutional committee.

Consent to participate/informed consent Informed consent was obtained from the patients for participation in the study.

Consent for publication Consent was obtained from the patients for publication of test results.

Conflict of interest The authors declare no competing interests.

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