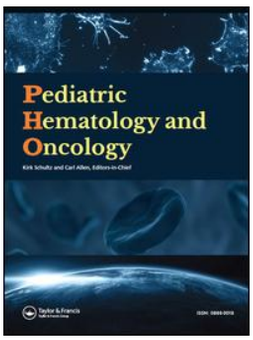

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BRIEF REPORT

A case of compound heterozygous hemoglobin Köln/ hemoglobin E in an Indian family

Navkirti Mittal[a], Akanksha Garg[a], Rajesh Kashyap[a], and Sarita Agarwal[b]

[a]Department of Hematology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India; [b]Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

ABSTRACT

Hemoglobin Köln, is the most common unstable hemoglobin variant worldwide, yet has only rarely been reported in Indians. Herein we report a case of coinheritance of Hb Köln and Hb E, which to the best of our knowledge has not been reported in the literature so far. The patient presented with mild symptoms of hemolysis with no previous history of blood transfusions.

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Hemoglobin Köln; hemoglobin E; unstable hemoglobin

Introduction

Unstable hemoglobinopathies are characterized by various mutations in the alpha or beta chains of hemoglobin (Hb) leading to its instability and precipitation. Hemoglobin Köln, is the most common unstable hemoglobin variant worldwide, yet has only rarely been reported in Indians.[1] It is a dominantly inherited disorder, resulting from a substitution of valine amino acid by methionine in codon 98 of the β globin gene (beta 98 [FG5] Val-Met) and is characterized by increased oxygen affinity, spontaneous denaturation of hemoglobin and varying clinical expression.[2] The most common hemoglobinopathies other than thalassemia in India are Hb S, Hb D and Hb E.[3] Herein we report a case of coinheritance of Hb Köln and Hb E, which to the best of our knowledge has not been reported in the literature so far.

Case report

A four-year old male child born of non-consanguineous parents was referred to our hospital with the complaints of weakness and easy fatigability of 1-year duration. There was no history of previous blood transfusions. Both the parents were asymptomatic and had no history of blood transfusions requirement. On physical examination the child had mild pallor and icterus. The liver was enlarged 3 cm below costal margin and the spleen was palpable 8 cm below costal margin. The skeletal, cardiovascular, respiratory and nervous systems examination did not reveal any abnormalities.

CONTACT Rajesh Kashyap rajkashyapmd@yahoo.co.in Department of Hematology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Rae Bairely Road, Lucknow 226014 Uttar Pradesh, India.
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Table 1. Hematological and biochemical profile of the patient.

Parameter	Normal range	Observed value
Hb (g/L)	115–135	97
TLC ($X10^9$/L)	4–10	4.2
Platelet count ($X10^9$/L)	150–1450	160
MCV (fl)	75–87	80.7
MCH (pg)	27–31	23.3
MCHC (g/dl)	31–37	26.6
Reticulocyte count (%)	1.0–2.0	6.7
Serum LDH (IU/L)	85–450	3902
Serum Bilirubin (mg/dl)	1.0–1.4	2.4

Abbreviations: Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular Hb; MCHC, mean corpuscular hemoglobin concentration; LDH, Lactate dehydrogenase level.

Complete blood count (CBC) revealed anemia with hemoglobin level (Hb) 97 g/L, mean corpuscular volume (MCV) 80.7 fl, mean corpuscular Hb (MCH) 23.3 pg, and mean corpuscular hemoglobin concentration (MCHC): 26.6 g/dl and a corrected reticulocyte count of 6.7%. The total leukocyte count and platelet counts were within normal range (Table 1). The peripheral smear showed red cell anisocytosis, poikilocytosis, polychromasia, target cell and basophilic stippling (Figure 1A). The serum lactate dehydrogenase level (LDH) and bilirubin levels were increased (Table 1). High performance liquid chromatography (HPLC) was performed on the patient's blood sample on Bio-Rad Variant II using beta thalassemia short program (Bio-Rad laboratories, California, USA). It showed two abnormal Hb peaks, one within the A2-window with a retention time (RT) of 3.72 min comprising 37.5% of the total Hb and the other peak within Hb C window with a RT of 5.04 min comprising 12.0% of the total Hb (Figure 1B). As per Bio Rad Library of Abnormal Hemoglobin version 3 the abnormal Hb in A2 window is most likely due to HbE or HbD-Iran and the peak within the Hb C window is possibly Hb Köln, which elute closest at that position (http://hemoglobins.bio-rad.com). Heat stability test with Tris-buffer solution at 50 °C depicted presence of flocculent precipitate after 1 hour of incubation in both patient and mother.

On family screening, father's CBC was normal and HPLC showed prominent peaks of HbA (49.2%) with abnormal Hb within HbA2-window (30.9%) with RT of 3.73 min depicting Hb E. Mother's hemogram also showed normal Hb and red cell indices and HPLC showed a major peak of HbA (52.1%) and another peak of an abnormal Hb within the C-window (15.8%) with RT of 5.07 min, possibly Hb Köln. For confirmation, Genomic DNA analysis of beta globin gene of the entire family was done, that confirmed the compound heterozygous state in the child for HbE (HBB: c.26 (G[C) and Hb Köln (HBB: c.98G [A) with the father being a heterozygote for HbA/HbE and the mother to be HbA/Hb Köln.

Discussion

Unstable hemoglobins account for approximately one-fifth of all the variant hemoglobins identified till date. Hb Köln also known as Hb Ube-1 is the frequent unstable hemoglobin and was described by Carrell et al. in 1966.[4] Hemoglobin Köln is most frequently seen in patients from Europe and the USA, and occasionally from Japan and South-East Asia.[5] Only a single case of Hb Köln has been reported from India by

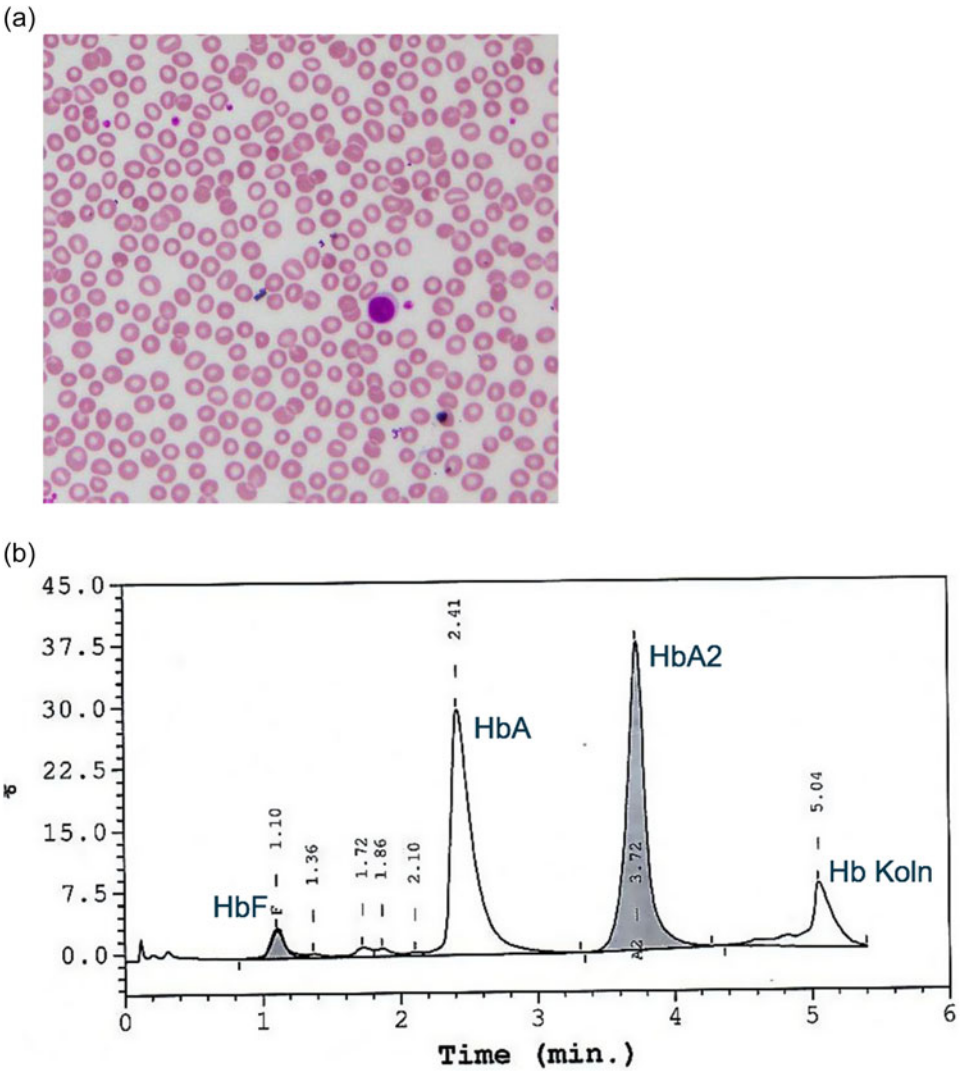

Figure 1. (A) Peripheral smear showing anisocytosis, poikilocyosis, hypochromia and polychromasia. (B) Cation-exchange HPLC analysis of patient's blood sample showed two abnormal Hb peaks, (i) A2-window – 37.5% with a retention time (RT) of 3.72 minutes; (ii) C-window – 12% with a RT of 5.04 minutes.

Warang et al., in a lady presenting with mild anemia, jaundice and hepatosplenomegaly.[1]

Patients with hemoglobin Köln present with hemolytic anemia, the clinical expression ranges from a compensated hemolytic state with exacerbations of hemolysis after exposure to drugs or during febrile episodes to a continuing and severe hemolytic anemia requiring splenectomy.[1,6,7] The instability of this hemoglobin variant is due to the substitution of valine by methionine at codon 98 resulting in heme loss through increased dissociation of tetrameric form of hemoglobin into dimeric form. The amino acid substitution also weakens the heme contact with globin, resulting in its precipitation in the red cells as Heinz bodies and subsequently hemolysis.[4,8]

Laboratory findings mainly reflect the hemolytic process: mild anemia, reticulocytosis, and presence of Heinz bodies, splenomegaly, elevated serum bilirubin and LDH levels.[1] On HPLC the mobility of Hb variants are expressed as retention times. The peak shape and retention time is useful in identifying different Hb Variants. However, the retention time is not unique to an individual Hb variant, Hb Köln shows an abnormal peak near to or in the C window and has to be differentiated from other Hb variants like Hb O Arab, Hb Constant Spring, Hb O Indonesia, HbA Genogi, and Hb Siriraj which also elute in the Hb C window and have similar retention times.[9] Hb Köln is the only unstable Hb in this group and can easily be detected on heat stability test. The heat stability test was crucial in this case as it helped identify the presence of unstable hemoglobin in both the patient and his mother and was later confirmed as Hb Köln using DNA analysis.

The hemolytic syndrome typically manifests in heterozygotes who present as autosomal dominant mode of inheritance however, sporadic cases also do occur.[1] Homozygosity for Hb Köln has never been described. Compound heterozygotes have rarely been reported. One case of a patient with both Hb Köln and beta thalassemia has been reported and this patient had only mild hemolytic anemia.[10] Patients with HbE trait are usually asymptomatic and have mild anemia. Co-inheritance of another hemoglobin variant commonly β-thalssemia trait modifies the clinical severity of the disease condition.[11] However, in our patient with Hb Köln co-inheritance of Hb E did not influence the course and severity of anemia. Nevertheless detection of Hb Köln in both the patient and other family members is important as these affected individuals may manifest severe hemolytic anemia later in life and may even require splenectomy. Splenectomy in these patients is associated with possible risk of thrombo-embolism.[6,7]

Disclosure statement

No potential conflict of interest was reported by the authors.

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