DOI: 10.7860/JCDR/2018/31302.11072

Case Report

Pathology Section

Combined Hereditary Spherocytosis and β-thalassemia trait: A Rare Co-existence

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ABSTRACT

Haemoglobinopathies are the commonest haemolytic disorders, prevalent in India and form a major bulk of patients in most of the haematology outpatient clinics. β-thalassaemia is the commonest inherited haemolytic anaemia and presence of β-Thalassaemia Trait (BTT) goes mostly undetected due to its asymptomatic clinical course. However, BTT should be diagnosed so as to conduct a genetic counselling and to prevent the number of births of affected children in turn reducing the financial burden on the affected family. Detection of combined haemolytic anaemia is on a rise due to better screening modalities in haemoglobinopathies. We hereby present two cases of combined BTT and Hereditary Spherocytosis (HS), and their clinical outcome.

Keywords: Haemoglobinopathies, Hepatosplenomegaly, Liquid chromatography, Osmotic fragility

CASE REPORT

First patient was an 18-year-old woman who came to the outpatient department of Internal Medicine with the complaints of fever and generalized weakness since two weeks. On physical examination, pallor was present along with icterus and hepatosplenomegaly. Second patient was a 52-year-old woman who had generalized weakness since 30 years of age and was being treated with haematinics until four years back when she was advised to get an ultrasonography of abdomen that revealed a grossly enlarged spleen, the size of which was increasing on serial imaging.

Complete blood counts of the patients are given in [Table/Fig-1]. Peripheral smear of both patients showed normocytic normochromic anaemia with presence of numerous uniform spherocytes with marked polychromasia [Table/Fig-2A-C,3A,B]. The white blood cells and platelets were reduced in number.

A work up for haemolytic anaemia was performed with Osmotic Fragility Test (OFT) and haemoglobin electrophoresis by High Performance Liquid Chromatography (HPLC) method. OFT without incubation showed normal fragility of the RBCs. Since a normal OFT did not correlate with presence of spherocytes on smear, OFT was repeated after incubating the samples for 24 hours. Increased fragility of RBCs were observed in the repeat OFT [Table/Fig-2D,3C]. This difference in OFT results before and after incubation was attributed to the presence of numerous polychromatophils. Meanwhile, the haemoglobin electrophoresis results revealed incidental elevation of isolated HbA2 and a diagnosis of β-thalassaemia trait was made [Table/Fig-2E,3D,4]. We could conclude that the presence of thalassaemic RBCs that were not detected on smear would have contributed to the normalization of OFT apart from presence of polychromatophils.

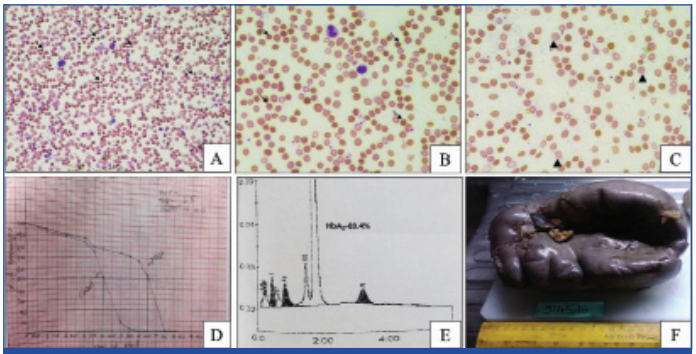
There was no family history of spherocytosis or thalassaemia in both the patients. The cases were concluded as HS with BTT. The patients were given symptomatic treatment and were followed up for nine months. Patients underwent therapeutic splenectomy, which on histopathological examination showed features of congestive splenomegaly [Table/Fig-2F,3E]. Second patient had associated pigmented gallstones [Table/Fig-3F]. Following surgery, the patients showed a relief of symptoms and are asymptomatic till date.

Parameter	First patient	Second patient
Haemoglobin (g/dl)	4.4	8.6
Reticulocyte count (%)	9.2	8.4
Mean cell haemoglobin concentration (g/dl)	35.1	31
Mean cell volume (fL)	72.4	74.8
Mean Corpuscular Haemoglobin (pg)	25.6	27.1
Red cell distribution width (%)	30	20.4
Red blood cell count (x10^{12}/L)	5.6	5.8
White blood cell count (x10^{9}/L)	1200	1300
Platelet count (x10^{9}/L)	81000	111000
Lactate Dehydrogenase (IU/L)	610	Not performed
Total Bilirubin (mg/dl)	3.35	1.46

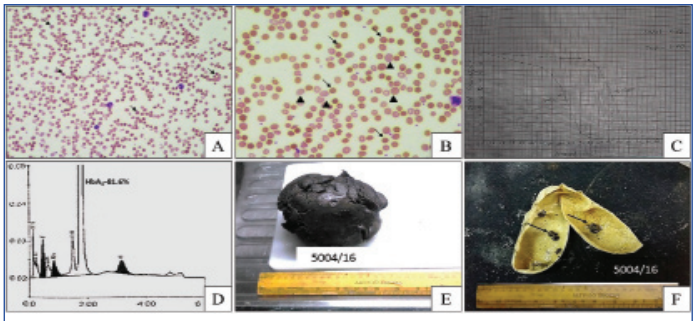
[Table/Fig-1]: Laboratory values.

DISCUSSION

HS is an inherited haemolytic anaemia whose prevalence is low in India [1]. The primary molecular lesion in HS is heterogeneous and involves membrane proteins like spectrin, ankyrin, band 4.2 and band 3 [2]. On the other hand, β-thalassaemia is



[Table/Fig-2]: (A) Peripheral smear with mild anaemia with numerous spherocytes (arrow) (Leishman stain; 100x); (B) Spherocytes (arrow) are uniform in size and shows absence of central pallor; (C) Reticulocytes (arrowhead) scattered amidst spherocytic RBCs. Note the presence of pancytopenia (Leishman stain; 200x); (D) Increased osmotic fragility of the patient's RBCs in OFT; (E) Haemoblobin electrophoeresis by HPLC with Elevated HbA2; (F) Grossly enlarged and congested spleen.



[Table/Fig-3]: (A) Peripheral smear shows normocytic normochromic anaemia with numerous spherocytes (arrow) (Leishman stain; 100x); (B) Uniform spherocytes (arrow) with moderate polychromasia (arrowhead). Note the decrease in the white blood cells and platelets (Leishman stain; 200x); (C) OFT showing increased fragility of patient's RBCs compared to normal control; (D) HPLC report with elevated HbA2 indicative of BTT; (E) Congestive splenomegaly; (F) Pigmented gall stones with thickened gall bladder wall.

Patient/ HPLC	HbA2 (%)	HbA1c (%)	HbA1a (%)	HbF (%)	HbA0 (%)
First Patient	3.8	3.9	1.2	2.9	83.4
Second Patient	4.8	5.2	0.9	2.6	81.6

[Table/Fig-4]: Haemoglobin electrophoresis results.

more common inherited haemolytic anaemia and the overall prevalence of BTT in India is 2.78% with a range from 1.48-3.64% [1]. The combination of HS with other inherited haemoglobinopathies such as sickle cell anaemia is reported in the literature and is rare [3]. However, the co-existence of HS and BTT is extremely rare and the reported cases on this combination are not many [2,4,5]. The clinical behavior and the interaction between these two diseases when they co-exist are still unclear. Here, we have described two such cases of co-existence of HS and BTT, the diagnosis of which rested on specialized laboratory findings.

HS is a familial haemolytic disorder secondary to red cell membrane defect and is characterized by a clinical triad of anaemia, intermittent jaundice and splenomegaly. A clinical response of betterment of anaemia following splenectomy is of diagnostic importance [3]. It has an autosomal dominant inheritance. β-thalassaemia is also a common inherited disorder and is considered the most common genetic disorder globally. In Indian subcontinent, the frequency of β–thalassaemia is high and the incidence ranges between 3% and 4% [1]. HS and BTT can coexist and the haemolytic anaemia secondary to this combination has got a variable severity as described by various authors [2,4-6]. Inheritance of HS has been reported in association with α-thalassaemia, β-thalassaemia and other enzyme deficiencies [7-9].

In BTT, there is loss of β-chains of globin leading to excess α-globin chains [10]. These excess α-globin get bound to the RBC cytoskeleton that results in structural reorganization, at times almost disrupting the skeletal network. If HS is associated with β-thalassaemia trait, the preexisting cytoskeletal abnormality of HS may get worsened due to further disruption by α-globin chains [2].

The major problem of HS is the circulating spherocytes that lack certain surface proteins. These spherocytes have decreased surface to volume ratio. Hence, the RBCs become rigid resulting in reduced flexibility. When these RBCs pass through the splenic vasculature, their reduced deformability results in trapping of cells and further loss of membrane by the action of splenic macrophages ultimately leading to haemolysis [2]. This property of spherocytes is the basis for the increased red cell osmotic fragility in HS. Whereas in thalassaemia, the RBCs have a reduced cell volume secondary to loss of globin chains and almost a near-normal surface area, as a result, they have an increased surface to volume ratio. Compared to HS RBCs, thalassaemic RBCs have increased resistance to osmotic fragility [10]. The combined effects of loss of cell surface area due to spherocytosis and decreased cell volume due to BTT results in cells with normal surface to volume ratio [2,10]. This can explain the wide range of clinical and laboratory findings encountered in patients having HS and BTT simultaneously. This could also explain the mild haemolysis seen in our cases.

In our patients, the presence of uniform spherocytes in the peripheral smears and reticulocytosis in a background of intermittent jaundice (noted in only one patient) and splenomegaly supported by increased osmotic fragility of RBCs resulted in diagnosis of HS. However, the presence of thalassaemic RBCs were not detected on smear. Increased RBC counts and occasional microcytic hypochromic RBCs were observed retrospectively only after the HPLC showed presence of BTT. The Red Blood Cell Distribution Width (RDW) in both of our cases were raised due to coexistence of spherocytic cells, polychromatophils and few microcytic hypochromic RBCs.

Causes of increased osmotic fragility are hereditary spherocytosis, autoimmune haemolytic anaemia, severe burns and snake bite. There is no history of burns or snake bite in our patients. Coombs test was negative which rules out a haemolytic pathology in both. Presence of large number of spherocytes in the peripheral smear with significant reticulocytosis pointed towards moderate HS [4]. Moderate HS is associated with a chronic haemolytic anaemia with moderately enlarged spleen and intermittent jaundice [4]. In our cases, the phenotypic expression of HS was mild due to silencing of HS by presence of BTT. However, in both patients, molecular studies to detect membrane and cytoskeletal protein abnormalities were not conducted.

CONCLUSION

HS has varied clinical manifestations as described in the literature. However, when HS has a significantly different clinical manifestation or shows laboratory findings that cannot be attributed to HS alone, a co-existence with other haemolytic anaemias should be kept in mind, especially in those countries where β-thalassaemia is observed frequently. The treating clinician should be aware of such combinations and the clinical diversities secondary to their co-existence for proper selection of treatment option. In both our patients, there was worsening of anaemia and surgical intervention was necessary to improve the clinical outcome.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 07, 2017**
Date of Peer Review: **Jul 17, 2017**
Date of Acceptance: **Aug 25, 2017**
Date of Publishing: **Jan 01, 2018**