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Original Research Paper

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EPB42 HEREDITARY SPHEROCYTOSIS WITH BETA THALASSEMIA TRAIT: CASE REPORT AND REVIEW OF LITERATURE.

Chintamani pathak MD, Professor, Department of pathology, VMMC & Safdarjang Hospital, New Delhi, India

Neha Aggarwal* MD, Senior Resident, Department of Pathology, VMMC & Safdarjang Hospital, New Delhi *Corresponding Author

Ritu bhatt MD, Senior Resident, Department of Pathology, VMMC & Safadarjang Hospital, New Delhi

ABSTRACT

Introduction: Hereditary spherocytosis (HS) is a inherited non-immune haemolytic anemia caused due to a spectrum of membrane protein defects that reduce the red cell deformability and Thalassemia is an autosomal recessive disorder characterised by a mutation or deletion of either the α or β- globin gene. The co- existence of HS and BTT is extremely rare and the reported cases on this combination are very few, with most of EPB42 related HS being reported from Japan. The clinical behaviour and the interaction between these two diseases when they co- exist are still unclear. Here, we have described one case of co-existence of HS and BTT diagnosed by specialised laboratory findings. **Case Presentation:** A five year old male child with severe anemia requiring multiple transfusions was referred to our hospital with complaints of fever and lethargy. He had pallor and jaundice with non-palpable liver and spleen. Complete blood count showed severe microcytic hypochromic anemia with Mentzer index=14.6. HPLC was performed which revealed increased HbA2 (4.1%). On Peripheral smear of the mother's blood many microspherocytes were seen . The chromatogram was normal for the mother. Molecular test to confirmed the presence of HS in the child in coexistence with BTT. **Discussion:** EPB42-associated hereditary spherocytosis is responsible for 40%-50% of hereditary spherocytosis in Japan. Few cases of EPB42- associated hereditary spherocytosis have been reported outside Japan while no case has been reported from India. Most of the cases with co-existing HS and hemoglobinopathy comprise of ankyrin, spectrin or band 3 mutations.

KEYWORDS : Hereditary spherocytosis, beta thalassemia, anemia

INTRODUCTION

Hereditary spherocytosis (HS) is a type of inherited non-immune haemolytic anemia caused due to a spectrum of membrane protein defects that reduce the red cell deformability, leading to increased hemolysis. It is characterised by variable degree of anemia, jaundice, splenomegaly, and cholelithiasis. [1] Prevalence of HS in the west is ~1:5000 to 1:10,000. However, the exact prevalence of HS in India is not known. [2,3] HS is classified on the basis of 5 genes which include α-spectrin (SPTA1), β-spectrin (SPTB), ankyrin (ANK1), band 3 (SLC4A1), and protein 4.2 (EPB42). These proteins are involved in the interaction between the membrane and the lipid bilayer. [4,5,6] The mutations seen in these genes differ in various regions of the world, hence, HS can easily be missed and overlooked [7,8,9]. The studies for molecular characterisation of HS in India are scarce. However, it has been seen that majority of cases of HS in India are caused due to ankyrin or β-spectrin mutations [10].

Thalassemia is an autosomal recessive disorder characterised by a mutation or deletion of either the α or β-globin gene, which causes blocked or complete inhibition of globin peptide chain synthesis. In β- thalasemia, due to insufficient β-globin peptide chains, α-globin peptide chains polymerise and form inclusion bodies that bind to the erythrocyte membrane skeleton and reduce deformability of the red cells, rendering them vulnerable to oxidative damage, leading to hemolysis and ineffective hematopoiesis [11]. β-thalassemia is traditionally classified into major, intermedia and trait based on genotype and clinical severity. β-thalassemia major is characterised by severe anemia requiring regular transfusions from an early age. β-thalassemia intermedia (BTI) lacks a specific genetic/molecular signature and diagnosis is largely based on an intermediate degree of clinical severity, with patients requiring transfusions at a later age than β-thalassemia major. β-thalassemia major & β-thalassemia intermedia are currently classified under Transfusion dependent Thalassemia. β- thalassemia trait (BTT) may have mild anemia and don't require treatment. Prevalence of BTT in India ranges from 1.48-3.64%. [12]

The combination of HS with other inherited haemoglobinopathies is rare. However, the coexistence of HS and BTT is extremely rare and the reported cases on this combination are very few, with most of EPB42 related HS being reported from Japan. [2] The clinical behaviour and the interaction between these two diseases when they co- exist are still unclear. [12] Three cases of combined HS with BTT have been described in India till now. [12,13] Here, we have described one case of co-existence of HS and BTT diagnosed by specialised laboratory findings.

Case Presentation

A five year old male child with severe anemia requiring multiple transfusions was referred to our hospital. The child presented with chief complaints of fever and lethargy. On physical examination, pallor and jaundice was present with non-palpable liver and spleen. Cardiopulmonary system examination was within normal limits. Serum LDH was raised with normal levels of Serum Iron, ferritin, TIBC, B12, and folate. Lab results are given in Table 1. Direct Coombs Test (DCT) and Indirect Coombs Test (ICT) was done to rule out possibility of immune hemolytic anemia and were negative. We received peripheral blood sample and bone marrow aspiration with bone marrow biopsy with requisition to rule out sideroblastic anemia

Complete blood count (CBC) [Table 1] showed severe microcytic hypochromic anemia with Mentzer index=14.6. On Peripheral smear, a microcytic hypochromic cells were seen admixed with normocytic normochromic cells (suggestive of recent transfusion) and moderate to severe degree of anisopoikilocytosis in the form of leptocytes, ovalocytes, target cells, tear drop cells and schistocytes with polychromasia (Fig 1a.) Platelets were raised with many large platelets and giant forms. The white blood cells were normal in total and differential count. Bone marrow examination showed erythroid hyperplasia with micronormoblastic reaction and normal myeloid and megakaryocytic maturation. (Fig 1f) No

sideroblasts were identified on Perl`s stain. Possibility of hemolytic anemia with thalassemia trait was considered. HPLC was performed (Fig 2a) which revealed increased HbA2 (4.1%). However, BTT is not associated with severe anemia and diagnosis of BTI was considered.

His parents were evaluated as well. Both parents were from Jammu and Kashmir, India with non-consanguineous marriage and negative family medical history and no history of blood transfusion. They were evaluated as well for the cause of anemia using routine haematological tests. After taking informed consent, 2 mL of peripheral venous blood (EDTA-K2 anticoagulant) was collected from the child's parents for CBC, reticulocyte count, and morphological investigations (parameters given in Table 2) as well as for High performance liquid chromatography (HPLC). Peripheral smear of the Father showed presence of mild anemia with presence of many microcytic hypochromic cells with many target cells (Fig 1b) and HPLC (Fig 2b) revealed raised HbA2 for the father (6.6%), suggesting BTT. On Peripheral smear of the mother's blood (Fig 1c), many microspherocytes were seen (5/hpf). The chromatogram parameters were within normal limits for the mother. (Fig 2c)

Osmotic fragility test was also performed on mothers blood which showed normal red cell fragility. Previous peripheral blood smears of the patient were reviewed which showed no evidence of microspherocytes. Fresh blood was also collected from the patient and smears prepared this time showed microcytic hypochromic cells (Fig 1d) with moderate to severe anisopoikilocytosis in the form of leptocytes, target cells, schistocytes as well as microspherocytes (4/hpf).

Table 1 And 2

	Rbc count	Hb	Hct	MCV	MCH	MCHC	RDW	Re-tic	PLT	TLC
Child	3.95	6.4	22.9	58	16.2	27.9	18.1	6.5%	4.1	5.3
Father	4.83	11.2	36.2	75	23.2	30.9	14.2		3.57	8.3
Mother	4.19	12.7	37.4	89	30.3	33.8	11.5		2.37	6.4

Lab Parameters	Value
LDH	2429
S. ferritin	70.5
S. Iron	135
TIBC	318
S. Vit B12	292
S.Folate	12

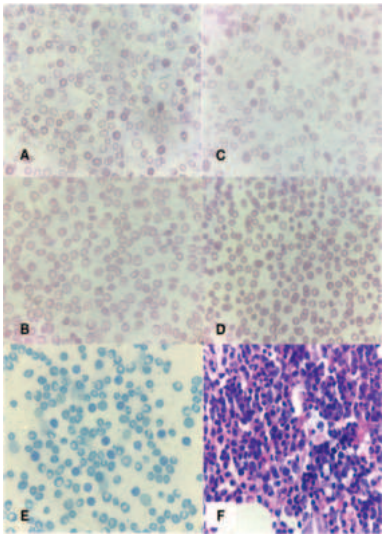

Figure 1

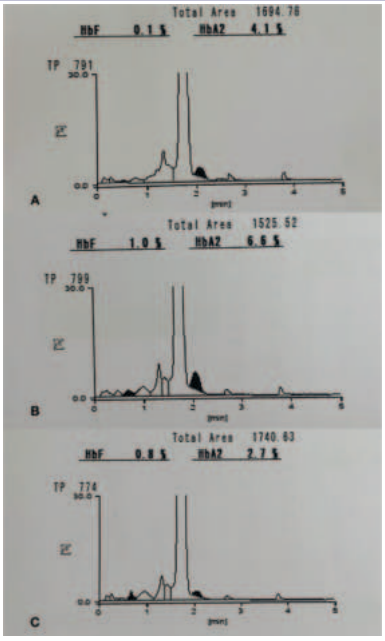

Figure 2

Genetic Tests

We sent the child for molecular test to confirm the presence of HS. The patient was analysed using next-generation (NGS) whole-exome sequencing, to identify mutated genes and sites which revealed a heterozygous splice site mutation in Intron 1 (c.10+91G>A) in Band 4.2 and classified as SPHT-5. Heterozygous frame shift mutation was also detected in Exon 2 of HBB gene (c.126_129delCTTT). Molecular test could not be performed for the mother as the patient was lost to follow up. The child was diagnosed as HS with concomitant BTT.

DISCUSSION

The initial evaluation of a person with hemolytic anemia includes: CBC and peripheral blood smear, Coombs test for immune hemolytic anemia; hemoglobin electrophoresis or HPLC and G6PD enzyme activity. Osmotic fragility testing or ektacytometry can help diagnose red cell membrane disorders. For non-immune hemolytic anemia, differential diagnosis that must be considered include other causes of hereditary hemolytic anemia like Hereditary elliptocytosis, stomatocytosis, or Southeast Asian ovalocytosis, Hemoglobinopathies, enzymopathies, Congenital dyserythropoietic anemia (especially type II).

EPB42-associated hereditary spherocytosis is responsible for 40%-50% of hereditary spherocytosis in Japan. In other populations, EPB42-HS accounts for 5% or less of HS. Few cases of EPB42-associated hereditary spherocytosis have been reported outside Japan including various parts of Europe and North America while no case has been reported from India to date due to molecular testing for HS. Most cases of HS in India have been attributed to β-Spectrin or Ankyrin mutations. Presently, at least 13 EPB42 variants have been described comprising of missense, nonsense, frameshift, splicing mutations and deletions. [2]

Most of the cases with co-existing HS and hemoglobinopathy comprise of ankyrin, spectrin or band 3 mutations. The clinical profile of hereditary spherocytosis with co-existing thalassemia varies from mild to moderate anemia with few cases reporting severe anemia. There have been cases of EPB42-associated hereditary spherocytosis with co-existing α-thalassemia which presented with moderate anemia not requiring transfusion. No case of EPB42-associated hereditary spherocytosis with co-existing β-thalassemia trait has yet been reported. Our case presented with severe

anemia and jaundice requiring multiple transfusions.

Case report by Maciag et al. in 2009 [14] identified 2 novel compound heterozygous mutations in Band 4.2 in a Polish individual. The patient had moderate degree of anemia (Hb 8.6 g/dl) due to hemolysis resulting from combined alpha thalassemia and HS, not requiring transfusion and not responding to hematinics. There was mild bilirubinemia (1.8mg/dl).

Xiaohong et al. [15] in China reported a case of alpha Thalassemia with HS type 3 (SCLC4A1) presenting with severe anemia (Hb 4.8 g/dl) requiring multiple transfusions from birth with raised bilirubin (2.26mg/dl).

Ming Chen et al. [11] reported a case of HS type 2 (SPTA1) with heterozygous beta Thalassemia presenting with severe anemia treated with multiple transfusions and gradually worsening splenomegaly. The patient underwent splenectomy which improved anemia and Hb was raised to 10.4 g/dl.

Uysal et al. in 2001 [16] reported case of a Turkish girl with HS and beta-Thalassemia major with severe anemia (Hb 4.9 g/dl) and failure to thrive. They were not able to analyse the membrane protein defect due to lack of facilities.

Aksoy et al. [17] found a Turkish patient with heterozygous beta Thalassemia and HS who presented with severe anemia (Hb 6.3 g/dl) from birth, raised bilirubin (1.6mg/dl) and cholelithiasis. BS Sridevi et al. [12] reported two cases from India with combined HS and Beta Thalassemia trait presenting with anemia (1st patient Hb: 4.4g/dl; 2nd patient Hb: 8.6g/dl) and raised bilirubin. First patient underwent splenectomy while the second patient had cholelithiasis. Both were relieved of symptoms post surgery.

Case reported by Sharma et al. [13] reported a Nepalese woman with mild anemia diagnosed as HS with Beta Thalassemia trait, though she was asymptomatic. Both the studies from India did not report genetic variant associated with HS.

To establish the severity of disease in HS the following tests have been recommended: Hemoglobin, reticulocyte count, Serum bilirubin, Transfusion history, Serum ferritin concentration to evaluate iron load status, Abdominal ultrasound examination to evaluate Spleen size and for detection of cholelithiasis. Those dependent on frequent transfusions require annual measurement of serum ferritin concentration and should be started on Iron chelation therapy if needed. [2]

Conventional methods, such as peripheral blood smear, osmotic fragility test have low sensitivity and specificity so HS is easily missed. With the rapid development of gene diagnostics, the detection of HS cases is increasing. Also, When clinical manifestations and laboratory results cannot be explained by a single haemolytic anaemia as was in our case, the possibility of combining with another haemolytic anaemia should be considered, especially in cases that are labelled as BTI.Thus, it is necessary to perform pedigree investigation and genetic analyses for a final diagnosis. [15]

Current guidelines suggest that HS can be diagnosed if there is family history of HS, and typical clinical and blood characteristics of HS or using molecular techniques [18]. This is suitable for HS patients with mild clinical symptoms or obscure clinical profiles [8]. A negative family history was one of the reasons for missing diagnosis in our case. Many mild HS patients have no obvious clinical symptoms in addition to combined HS and BTT which show a variable clinical profile, making it difficult to diagnose the disease using conventional methods. This was particularly true in case of the mother who was asymptomatic but peripheral blood showed spherocytes with a negative OFT. Additionally, HS could only diagnosed in the child due to genetic tests. Initial periphery blood smears from the patient did not show presence of spherocytes, likely due to multiple transfusions for severe anemia. Additionally, MCHC values were reduced in the patient, while in classical HS cases MCHC is raised, therefore the diagnosis was missed. [15]

In some cases, mutations that can be inherited and lead to severe HS, genetic counselling and prenatal diagnosis can play an extremely important role in prenatal and postnatal care. Thus, if ≥2 members of a family with HS are found to have the same mutation in the same gene, this suggests that the mutation site is hereditary. This is important when studying the molecular genetic mechanisms of HS and assessing the risk of HS for other members or descendants of the family, and can guide the future of precision medical care. [8]

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