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Karnataka Paediatric Journal

Article in Press

Case Report

Hereditary spherocytosis with preserved eosin-5-maleimide binding

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Received: 09 December 2024
Accepted: 14 January 2025
Epub Ahead of Print: 19 February 2025
Published:

DOI
10.25259/KPJ_54_2024

Quick Response Code:

ABSTRACT

Hereditary spherocytosis (HS) is a common congenital hemolytic anemia caused by genetic mutations affecting red blood cell (RBC) membrane proteins and is usually inherited in an autosomal dominant manner. The diagnosis of HS is based on clinical and laboratory features, but traditional diagnostic methods have limitations in sensitivity and specificity. This study presents the clinical course and diagnostic challenges of a 16-year-old girl with HS complicated by vitamin B12 deficiency. She presented with a one-month history of dyspnea, pallor, jaundice, and hepatosplenomegaly, with signs of cardiac failure. Initial investigations revealed severe anemia with a few spherocytes and macro-ovalocytes on a peripheral smear. Despite a negative eosin-5-maleimide (EMA) binding test, due to the presence of spherocytes, an elevated mean corpuscular hemoglobin concentration (MCHC), and a strong family history, raised the suspicion of HS. Genetic mutation analysis confirmed Ankyrin 1 (ANK-1) gene mutation, consistent with HS. The patient was concurrently diagnosed with vitamin B12 deficiency, which had exacerbated the anemia. This case highlights the diagnostic challenges in hereditary spherocytosis, particularly when associated with nutritional deficiencies. Early diagnosis and appropriate management, including genetic testing and nutritional supplementation, are critical for optimal patient outcomes.

Keywords: Eosin-5-maleimide-negative hereditary spherocytosis, Phenotype genotype difference in hereditary spherocytosis, Splenectomy, *ANK 1* mutation

INTRODUCTION

Hereditary spherocytosis (HS) is a common haemolytic anaemia resulting from gene mutations encoding red blood cell (RBC) membrane proteins. This disorder is predominantly inherited in an autosomal-dominant manner. The diagnosis is based on a clinical history of anaemia (easy fatigability and dyspnoea), jaundice, hepatosplenomegaly in the background of strong family history, the presence of spherocytes on a peripheral blood smear, elevated mean corpuscular haemoglobin concentration (MCHC), increased osmotic fragility and decreased eosin-5-maleimide (EMA) binding. However, these diagnostic methods have variable sensitivity and specificity.[1]

Recent studies have improved the diagnostic accuracy for HS by employing methods based on the principle of flow cytometry, which is considered the gold standard for confirming HS. While the EMA binding test has a high specificity of 98%, its sensitivity is 93%, and there is a possibility of false-negative results.[2]

CASE REPORT

A 16-year-old girl, previously normal, well grown, weight - 44 kg (between 25th and 50th centile) and height 167 cm (between 90 and 97th centile), presented with a 1-month history of dyspnoea. Clinical examination revealed pallor, icterus, hepatosplenomegaly (liver span 8 cm and spleen size 3 cm below left costal margin) and signs of cardiac failure. Initial laboratory investigations showed low haemoglobin levels of 2.9 g/dL, total counts of 7640/mm^3, with differential counts - neutrophils - 58.9%, lymphocytes 32.5%, eosinophils - 2.5%, monocytes - 6.1%, platelet count - 1.08 lakh, reticulocyte count of 0.21% and a peripheral blood smear which was negative for atypical cells but demonstrated few spherocytes and predominant macro-ovalocytes. Direct Coombs test was negative. Total bilirubin was 2.42 mg/dL, with direct bilirubin being 0.44 mg/dL. Further evaluation revealed that the patient was a picky eater; subsequent testing confirmed low vitamin B12 levels <159 pg/mL (160–950 pg/mL). The patient was initiated on vitamin B12 and folic acid supplementation. The child received a packed RBC transfusion for her acutely decompensated state and was started with Vitamin B12 supplements. Initially, IV B 12 of 1000 mcg/day later switched over to oral after 1 week. Platelet counts improved to more than 1.5 lakh after 72 h of B12 supplementation.

Osmotic fragility test results showed a normal pattern with haemolysis that began at 0.4% and ended at 0.1%, with median corpuscular fragility being 4.1% (normal range 4–4.5%).

Flow cytometric analysis using EMA showed a mean fluorescence intensity (MFI) of 8609 versus a control of 8429. The patient's MFI (8609) is slightly higher than the control (8429), with only a 2.13% increase in MFI percentage difference (MFI Patient – MFI Control/MFI control) × 100, which is not consistent with HS.

However, due to the elevated mean corpuscular hemoglobin concentration of 34.5 g/dl (MCHC>33 g/dL), the presence of spherocytes, and the family history of the mother undergoing splenectomy at 12 years of age due to a blood disorder (details not available), diagnosis of hereditary spherocytosis was considered.

Whole exome sequencing was performed, which identified an ANK1 gene mutation (*ANK1* (NM_000037.4), Intron 20, c.2296-2A>G,) which was a heterozygous pathogenic autosomal dominant mutation confirming the diagnosis of hereditary spherocytosis type 1.

The patient is currently under follow-up, continuing oral vitamin B12 supplementation with a good clinical response. Haemoglobin levels improved following a single transfusion and Vitamin B12 and folic acid supplementation, now stabilised between 10 and 11 g/dL, with a healthy reticulocyte count (6–7.3%) and she is doing well in the 3-month follow-up.

Further management of the child will include immediate family screening and close follow-up. As the child is currently thriving and responding well to oral treatment, splenectomy is not indicated. However, if the need for repeated blood transfusions arises or if growth retardation occurs, splenectomy may be considered.[3]

DISCUSSION

HS is a common cause of congenital haemolytic anaemia, characterised by clinical features such as anaemia, jaundice and splenomegaly. The pathophysiology of HS involves defects in the RBC membrane cytoskeleton, leading to reduced membrane stability and the formation of spherocytes, which are prone to premature destruction in the spleen.

HS can be diagnosed at any age, from the neonatal period to adulthood.[4] In mild cases of HS, patients typically exhibit compensated haemolysis without anaemia. However, in moderate–to-severe cases, patients experience incompletely compensated haemolysis, leading to anaemia. These more severe cases are also characterised by mild-to-moderate splenomegaly, haemoglobin levels ranging from 7 to 9 g/dL and reticulocytosis of 10% or higher.[5]

HS can clinically manifest as mild, moderate and severe clinical forms. This patient exhibited a mild phenotype, probably explaining her being asymptomatic till this presentation. However, the concomitant Vitamin B12 and probably folic acid deficiency led to clinical decompensation. The patient's mother, who is presumed to have the same condition, underwent a splenectomy at approximately 12 years of age. In contrast, our index case was not diagnosed until 14 years of age and presented with symptoms primarily due to associated nutritional deficiencies. This highlights the variability in genotype and phenotype expression, even with the likely same genetic mutation in both mother and child. The father has been clinically evaluated, remains asymptomatic and shows no evidence of splenomegaly.

Conventionally, the diagnosis of HS has relied on osmotic fragility testing using NaCl or glycerol, which assesses the vulnerability of RBCs to lysis in hypotonic solutions. However, these tests have limited sensitivity and specificity, particularly in mild cases of HS. Advances in diagnostic techniques have led to the development of more specific assays, such as the EMA binding test, which directly targets the molecular defect in HS. EMA is a fluorescent probe that binds to transmembrane proteins, including band 3, Rh protein, Rh glycoprotein and CD47.[6] These proteins are reduced in RBCs from patients with HS, resulting in decreased fluorescence intensity. In addition, defects in cytoskeletal proteins such as spectrin and protein 4.2 can

indirectly reduce EMA binding, likely due to alterations in the conformation of the band 3 protein.

The EMA binding test has become a valuable diagnostic tool due to its high specificity and relative ease of use. A significant decrease in MFI (often >10–15% reduction compared to control) is seen in HS due to reduced binding of EMA to band 3 and other proteins. Our patient MFI showed a 2.13% increase which is not consistent with HS. Patients with mild forms of HS may have near-normal EMA binding due to minimal reduction in membrane protein defects (such as spectrin, ankyrin or band 3 protein). The fluorescence reduction may fall within the normal range, leading to a false-negative result.[3] Conditions such as iron deficiency anaemia, vitamin B12 deficiency or folate deficiency can alter red cell membrane characteristics, potentially masking the reduced EMA binding in HS.[7] Other occasions where false-negative results have been seen include in conditions with co-existent thalassemia and other haemoglobinopathies which can modify red cell membrane properties,[7] mutations in less common genes, such as those associated with stomatin-deficient HS or other membrane protein abnormalities, may not significantly affect band 3 protein,[5] which is the target of the EMA test, delayed shipment in which tests are performed on stored samples >48 h and analytical errors of laboratory.[3]

Therefore, when clinical suspicion is high but EMA results are negative, additional testing such as genetic analysis may be warranted to confirm the diagnosis.[8]

Overall, the integration of clinical, laboratory and genetic data is essential for the accurate diagnosis and management of HS, especially in cases complicated by overlapping conditions such as Vitamin B12 deficiency or iron deficiency, which can exacerbate the anaemia and complicate the clinical picture as in our patient. A repeat EMA test done after correction of nutritional deficiencies with an appropriate reticulocyte response may likely give a correct EMA result.

Lessons learned

Diagnosis of haemolytic anaemia like hereditary spherocytosis can be challenging due to various factors such as the type of mutations involved, genotype-phenotype discrepancy, and compounding nutritional deficiencies. Hence, a thorough clinical workup and, if required, genetic workup is essential to manage a child with such disorders. Eosin-5-maleimide, though has high specificity, can still miss certain kinds of hereditary spherocytosis which can be diagnosed with other methods, such as mutational analysis.

CONCLUSION

This case highlights the diagnostic challenges in HS, particularly when complicated by nutritional deficiencies, despite the limitations of standard diagnostic tests such as EMA binding, a comprehensive clinical and genetic approach led to the diagnosis. The patient's mild HS phenotype, compounded by Vitamin B12 deficiency, underscores the importance of considering compounding aetiologies in patients presenting with haemolytic anaemia. Early diagnosis and appropriate management, including genetic testing and nutritional supplementation, are critical for optimal patient outcomes.

Ethical approval: Institutional Review Board has waived the ethical approval for this study.
Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent.
Financial support and sponsorship: Nil.
Conflict of interest: There are no conflicts of interest.
Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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How to cite this article: Bhargavi S, Jeniffer VN, Ramu SA. Hereditary spherocytosis with preserved eosin-5-maleimide binding. Karnataka Paediatr J. doi: 10.25259/KPJ_54_2024