Acta Scientific PAEDIATRICS (ISSN: 2581-883X)

Volume 4 Issue 12 December 2021

Case Series

Hereditary Non Spherocytic Hemolytic Anemia: A Series of Rare Hereditary Red Cell Enzymopathies

Meena Sivasankaran[2]*, Vamsi Krishna Reddy[1], Ravikumar Thangadorai[1], Vimal Kumar[2], Deenadayalan Munirathnam[2]

[1]Department of Paediatrics, Kanchi Kamakoti CHILDs Trust Hospital, Numgambakkam, Chennai, India

[2]Department of Paediatric Haemato-oncology, Kanchi Kamakoti CHILDs Trust Hospital, Numgambakkam, Chennai, India

***Corresponding Author:** Meena Sivasankaran, Department of Paediatric Haemato-oncology, Kanchi Kamakoti CHILDs Trust Hospital, Numgambakkam, Chennai, India.

Received: March 01, 2021

Published: November 29, 2021

Abstract

Hereditary red cell enzyme disorders are a group of non-immune/non-spherocytic hemolytic anaemia. It occurs due to defect in the genes encoding red blood cell enzymes. Glucose 6 phosphate dehydrogenase (G6PD) is the most common enzyme deficiency. However there are number of other enzyme defect, leading to non-spherocytic hemolytic anemia of variable severity. We describe a series of 4 children with rare RBC ezymopathies.

Keywords: Enzymopathies; Non-immune; Non-spherocytic; Hemolytic Anaemia

Key Point

Child with chronic non spherocytic/non immune hemolysis evaluate for enzymopathies.

Introduction

Hereditary red blood cell enzymopathies are a group of Non-Spherocytic Hemolytic Anaemia (HNSHA). They occur due to a defect in genes encoding red blood cell (RBC) enzymes. It affects RBC cellular metabolism, mainly anaerobic glycolysis, the hexose monophosphate shunt, glutathione metabolism, and nucleotide metabolism [1]. Glucose 6 Phosphate Dehydrogenase (G6PD) deficiency is the most common enzyme disorder. However there are number of other enzymes defect, leading to non-spherocytic hemolytic anaemia of variable severity, ranging from mild to fatal anemia dependent on the severity of enzyme deficiency. Diagnosis is mainly based on detection of reduced specific enzyme activity and molecular characterization of the defect on the DNA level [2].

Here, we describe a series of 4 children with rare hereditary red cell enzymopathies.

Patients and Results

Case 1

An 11-month old girl, second born to consanguineous parents presented to us at 11 months of life with severe pallor and features of congestive cardiac failure. She was noted to have moderate splenomegaly. She had history of neonatal hyperbilirubinemia needing exchange transfusion at day 3 of life and had episodic drop of hemoglobin and required blood transfusion every 3 months at 3, 6 and 9 months of life. Blood picture at each episode was suggestive of coombs negative hemolytic anemia and indirect hyperbilirubinemia. Hemoglobin at presentation was HB-2.1 g/dl. Peripheral smear was suggestive of polychromasia and bite cells. Reticulocytosis was noted (39%). Heinz body preparation was positive suggestive of oxidant injury (Figure 1 and 2). Direct coombs test was

negative. Hemoglobin electrophoresis was normal. Bone marrow examination was done showed normal trilineage hematopoiesis with erythroid hyperplasia.

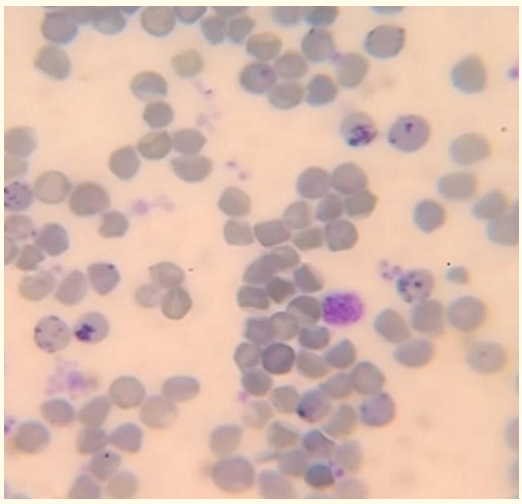

Figure 1: Supra vital stain showing Heinz bodies.

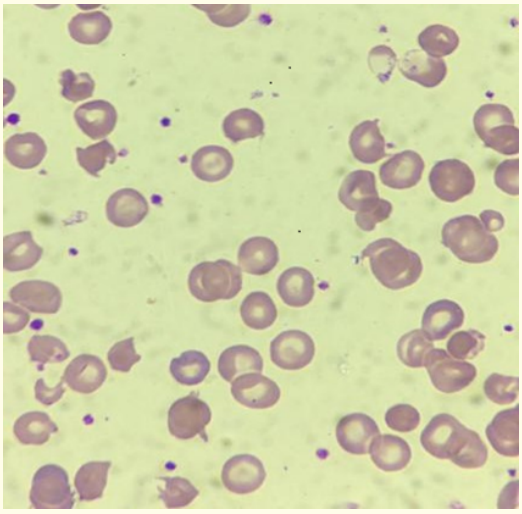

Figure 2: Peripheral smear showing polychromasia, blister cell and bite cell suggestive of oxidative hemolyosis.

Gene sequencing was suggestive of homozygous missense variation in exon 12 of GPI gene (chr19:g:34884949G; Depth:58x) resulting in the amino acid substitution of histidine for arginine at codon 358 (p.Arg358His; ENST00000415930.3). This GPI gene variant was classified as pathogenic variant.

Case 2

An 11 year old boy born to consanguineous parents, presented to us with easy fatigability and severe pallor. He had a history of neonatal hyperbilirubinemia required exchange transfusion. He also had history of episodic drop in hemoglobin with high colored urine during febrile illness and required blood transfusion. On examination he had severe pallor, icterus and moderate splenomegaly.

Hemoglobin at presentation was 5.2 gm/dl. Peripheral smear showed howell jowell bodies, polychromatophils and echinocytes. Reticulocyte count was 11%. ICT and DCT were negative, Heinz body preparation and osmotic fragility tests were negative. Isopropranolol precipitation test was positive.

Gene sequencing was suggestive of A homozygous missense variation in exon 6 of the GPI gene (chr19:g.34868652G>T: Depth: 121x) that results in amino acid substitution of leucine for valine at codon 178(p.Val178Leu: ENST00000415930.3). This GPI gene variant was classified as variant of unknown significance.

Case 3

A 9-year-old boy first born to consanguineous parents presented to us with acute febrile illness. He had history of neonatal unconjugated hyperbilirubinemia and required exchange transfusion. He received multiple transfusions (six blood transfusions) during the febrile episode. On examination noticed to have pallor and splenomegaly. Blood investigation revealed low hemoglobin (6 g/dl), Indirect hyperbilirubinemia (3.5 mg/dl), and reticulocytosis (9%). Peripheral smear showed polychromasia, schistocytes and elliptocytes. Heinz body preparation, osmotic fragility test and isopropranolol precipitation tests were negative.

Gene sequencing was done suggestive of A homozygous nonsense variation in exon 4 of the AKI gene (chr9:130635115T>A; Depth: 75x) that results in a stop codon and premature truncation

of the protein at codon 21 (p.Lys21Tre; ENST00000373176.1). This AKI variation is classified as a pathogenic variant.

Case 4

A 9-month-old girl infant 3rd born to consanguineous parents referred to us in view of low hemoglobin. She had neonatal hyperbilirubinemia and required exchange transfusion. She had significant family history (elder sibling had neonatal hyperbilirubinemia required exchange transfusion, died at 1.5 year of age due to jaundice). She had previous history of blood transfusions at 3 and 5 months of age. Clinically child had frontal bossing and splenomegaly.

Baseline investigations showed anemia (HB-5 g/dl), Indirect hyperbilirubinemia (5 mg/dl) and reticulocytosis (12%). Direct coombs test was negative and Osmotic fragility test was normal.

Gene sequencing was done suggestive of A homozygous missense variation in exon 4 of the PKLR gene(chr1:g.155295547T>C: Depth 270x) that results in the amino acid substitution of aspartic acid for asparaginase at codon 133 (p.Asn133Asp; ENST0000034741.6). This PK gene variant was classified as pathogenic variant.

Discussion

Hereditray rare red cell enzymopathies pose a diagnostic challenge and patients may undergo repeated unsuccessful investigations over the years. Enzymopathies causes normocytic/marocytic anemia with signs of hemolysis that is increased plasma levels of bilirubin, lactate dehydrogenase, and high reticulocyte count. The diagnosis of HNSHA due to a red blood cell enzymopathy generally a diagnosis that is based on exclusion: a negative direct coombs test, a normal osmotic fragility/cryohemolysis test, no specific morphological abnormalities, and no evidence for an abnormal hemoglobin. Diagnosis of a red blood cell enzymopathy requires demonstration of reduced enzymatic activity and confirmation of the diagnosis on the DNA level [3,4].

Hereditary red blood cell enzymopathies are disorders arising from mutations in genes coding for red cell metabolic enzymes. Deficiency of these enzymes leads to impair cellular energy and/or increase the levels of oxidative stress and leading to premature removal in the spleen and, consequently, decreased red blood cell survival [1].

G6PD deficiency is the most common enzyme deficiency in hexose monophosphate shunt and glutathione metabolism. It is inherited in an X linked recessive manner [5]. Most other enzyme disorders are inherited in an autosomal recessive form with hemolysis occurring only in homozygous or compound heterozygous individuals [1].

The second most prevalent enzyme disorder of anaerobic glycolysis (HMP shunt) is pyruvate kinase (PK) deficiency. Pyruvate kinase is a key glycolytic enzyme that catalyses the trans phosphorylation from phosphoenolpyruvate (PEP) to ADP, yielding pyruvate and ATP. Most affected individuals have enzyme activity less than 40%. The clinical features of PK deficiency are highly variable, ranging from a fully compensated hemolysis to severe transfusion-dependent hemolytic anemia. The severity of anemia usually stable but may worsen during infections or any other physiological stress [6].

The third most frequent glycolytic enzyme disorder is glucose-6-phosphate isomerase deficiency (GPI). In most affected individuals, residual GPI activity is less than 25%. Clinical features in GPI activity range from mild-to-severe hemolytic anemia. Hydrops fetalis appears to occur more often in GPI deficiency than in other enzymopathies. This anemia caused by GPI deficiency can develop into a hemolytic crisis as a result of exogenous oxidant agents like infections or drugs. This is a consequence of the reduction of the antioxidant enzymatic system due to GPI deficiency GPI deficiency may also be associated with non-hematological symptoms, in particular neurological impairment or mental retardation [7,8].

Adenylate kinase (AK) is a ubiquitous enzyme which catalyses the inter conversion of adenine nucleotide. Clinically, AK deficiency is associated with mild to severe anemia with hepato-splenomegaly in almost all the reported cases. Psychomotor impairment is reported in few of the cases [9].

There are a number of enzymes involved in nucleotide metabolism. The most important ones are pyrimidine-5- nucleotidase (pyrimidine metabolism) and adenylate kinase and adenosine deaminase (purine metabolism). Other enzyme deficiencies like glutamate cysteine ligase, glutathione synthetase, and glutathione reductase are very rare. Defects of these enzymes are associated with HNSHA. Generally, disorders of these enzymes cause hemolysis only under conditions of increased levels of oxidative stress [10].

The treatment of red blood cell enzymopathies remains mainly supportive. Prevention plays a major role in glucose-6-phosphate dehydrogenase deficiency. Splenectomy is indicated in severe cases of pyruvate kinase and glucose phosphate isomerase deficiency. Other deficiencies of the glycolytic pathway are rare and their treatment has not been codified [1,11].

In this study all four cases born to consanguineous parents and had neonatal hyperbilirubinemia requiring exchange transfusion and later life received multiple blood transfusions especially at the time of acute febrile illness. All children evaluated step by step showed low hemoglobin (normocytic/macrocytic anemia) and elevated reticulocyte counts. Direct cooms test was negative. Hemoglobin electrophoresis and osmotic fragility tests were done normal. Next generation sequencing helped us in diagnosis in these children. Two children had Glucose-6-Phosphate Isomerase deficiency (GPI), one had Adenylate Kinase (AK) deficiency and one had Pyruvate Kinase (PK) deficiency.

Conclusion

A child with non spherocytic/non-immune hemolysis anemia needs to be evaluated for enzymopathies. Although no definitive therapy is not available at present, accurate genetic diagnosis of these conditions are important for achieving a definitive diagnosis, appropriate genetic counselling, prognosis and treatment. Management is mainly supportive. Splenectomy indicated in severe cases of pyruvate kinase and glucose phosphate isomerase deficiency. A novel treatment including enzyme activator is now under development and this might provide a new option for patients with severe phenotypes.

Bibliography

1. Dreyfus JC and Kahn A. "Red cell enzymopathies: molecular mechanisms". *Clinical Biochemistry* 17.6 (1984): 331-340.
2. Van Wijk R and van Solinge WW. "Disorders of red cells resulting from enzyme abnormalities" (2010).
3. Koralkova P., *et al*. "Rare hereditary red blood cell enzymopathies associated with hemolytic anemia–pathophysiology, clinical aspects, and laboratory diagnosis". *International Journal of Laboratory Hematology* 36.3 (2014): 388-397.
4. Kedar PS., *et al*. "Molecular diagnosis of unexplained haemolytic anaemia using targeted next-generation sequencing panel revealed (p. Ala337Thr) novel mutation in GPI gene in two Indian patients". *Journal of Clinical Pathology* 72.1 (2019): 81-85.
5. Frank JE. "Diagnosis and management of G6PD deficiency". *American Family Physician* 72.7 (2005): 1277-1282.
6. Zanella A., *et al*. "Red cell pyruvate kinase deficiency: molecular and clinical aspects". *British Journal of Haematology* 130.1 (2005): 11-25.
7. Manco L., *et al*. "Hereditary nonspherocytic hemolytic anemia caused by red cell glucose-6-phosphate isomerase (GPI) deficiency in two Portuguese patients: clinical features and molecular study". *Blood Cells, Molecules, and Diseases* 60 (2016): 18-23.
8. Warang P., *et al*. "Hereditary non-spherocytic hemolytic anemia and severe glucose phosphate isomerase deficiency in an Indian patient homozygous for the L487F mutation in the human GPI gene". *International Journal of Hematology* 96.2 (2012): 263-267.
9. Abrusci P., *et al*. "Erythrocyte adenylate kinase deficiency: characterization of recombinant mutant forms and relationship with nonspherocytic hemolytic anemia". *Experimental Hematology* 35.8 (2007): 1182-1189.
10. Arya R., *et al*. "Hereditary red cell enzymopathies". *Blood Review* 9 (1995): 165-175.
11. Niizuma H., *et al*. "Splenectomy resolves hemolytic anemia caused by adenylate kinase deficiency". *Pediatrics International* 59.2 (2017): 228-230.

Volume 4 Issue 12 December 2021