REFERENCES

1. Tambourgi DV, Morgan BP, de Andrade RM, et al. Loxosceles intermedia spider envenomation induces activation of an endogenous metalloproteinase, resulting in cleavage of glycophorins from the erythrocyte surface and facilitating complement-mediated lysis. Blood. 2000; 95:683-91.
2. Naik R. Warm autoimmune hemolytic anemia. Hemato-Oncol Clin North Amer. 2015;29:445-53.
3. Monzon C, Miles J. Hemolytic anemia following wasp sting. J Pediatr. 1980;96:1039-40.
4. Biswas S, Chandrashekhar P, Varghese M. Positive hemolytic anemia due to insect bite. Oman Med J. 2007;22:62-3.

Hereditary Non-Spherocytic Hemolytic Anemia (HNSHA): Four Children with Rare Hereditary Red Cell Enzymopathies

Hereditary red blood cell (RBC) enzymopathies, a group of non-immune, non-spherocytic hemolytic anemias, occur due to a defect in the genes encoding red cell enzymes. Glucose-6-phosphate-dehydrogenase (G6PD) deficiency and pyruvate kinase (PK) deficiency are the commonly reported red cell enzymopathies. Herein, we describe four children with rare red cell enzymopathies [1].

An 11-month-old girl child, the product of consanguineous marriage, presented with pallor, splenomegaly, and cardiac failure. Investigations were suggestive of hemolytic anemia (**Table I**). Direct Coombs test (DCT), hemoglobin electrophoresis, osmotic fragility test (OFT), isopropanol stability tests for unstable hemoglobins, HbH preparation, and G6PD assay were non-contributory. She had a history of neonatal hyperbilirubinemia needing exchange transfusion followed by a history of blood transfusion at 3 and 6 months of age. Next-generation sequencing (NGS) detected a homozygous missense variation in exon 12 of the *glucose-6-phosphate-isomerase (GPI)* gene.

An 11-year-old boy born to a consanguineous marriage presented with severe pallor, and splenomegaly. Laboratory workups were suggestive of Coombs negative hemolytic anemia (**Table I**). He had a history of neonatal hyperbilirubinemia warranting an exchange transfusion, followed by global developmental delay with sensory neural hearing loss (neurological sequelae of bilirubin encephalopathy). He also had a history of blood transfusion in infancy. A homozygous missense variant in exon 6 of the GPI gene was detected by NGS, which has also been previously reported to cause neurologic impairment and HNSHA [2].

A 9-year-old boy, product of consanguineous marriage, presented with severe pallor and splenomegaly. He had a history of exchange transfusion for hyperbilirubinemia in the neonatal period and also needed repeated blood transfusions for anemia. Laboratory workup (**Table I**) did not reveal a cause for hemolysis. NGS detected a homozygous nonsense variation in exon 4 of the *AK1* (adenylate kinase*)* gene, previously reported to cause hemolytic anemia [3].

A 9-month-old girl, product of consanguineous marriage, presented with severe pallor and splenomegaly. She had neonatal hyperbilirubinemia and required exchange transfusion. Her elder sibling had a history of neonatal exchange transfusion; he died at 1.5 years of age due to severe anemia with jaundice. Investigations were suggestive of Coombs negative hemolysis (**Table I**). A homozygous missense variant in exon 4 of the PKLR (pyruvate kinase L/R) gene was detected by NGS, which can lead to HNSHA.

Table I Clinical Profile and Laboratory Work-up of the Children With Red Cell Enzymopathies

Characteristics	*Case 1*	*Case 2*	*Case 3*	*Case 4*
Age at presentation	11 mo	11 y	9 y	9 mo
Clinical features[a]	-	Developmental delay	-	-
Transfusion history	Once in 3 mo	During acute febrile illness	During acute febrile illness	Once in 3 mo
Consanguinity	3rd degree	3rd degree	3rd degree	2nd degree
Hb (g/dL), MCV (fL), Reticulocyte count (%), Indirect bilirubin (mg/dL)	2.1, 120, 39%, 3	5.2, 112, 11%, 4.5	6, 92, 7%, 3.9	5, 89, 12%, 3.5
Peripheral smear	Macrocytes, bite cells, polychromasia	Macrocytes, polychromasia	Polychromasia, elliptocytes	Polychromasia
Heinz body preparation	Positive	Negative	Negative	Negative

[a]*Pallor, icterus and splenomegaly was present in all children. Other investigations (DCT: direct Coombs test, OFT: osmotic fragility test, HPLC: high performance liquid chromatography, HBH preparation (for alpha thalassemia), Isopropanol stability test (for unstable hemoglobinopathies), G6PD:Glucose 6 phosphate Dehydrogenase) were normal for all children. HB: hemoglobin, MCV: mean corpuscular volume. Bone marrow examination showed erythroid hyperplasia in all children.*

All children are presently receiving nutritional supplementation and intermittent transfusions. RBC enzymopathies arise from mutations in genes coding for RBC metabolic enzymes. Deficiency of these enzymes leads to impaired cellular energy and/or increases the levels of oxidative stress, leading to premature removal of RBCs in the spleen and decreased red blood cell survival [1]. There are enzymes other than G6PD and PK, which are involved in nucleotide metabolism. The important ones are pyrimidine-5-nucleotidase (pyrimidine metabolism) and adenylate kinase and adenosine deaminase (purine metabolism) [1]. The clinical features of enzymopathies are highly variable, ranging from fully compensated hemolysis to severe transfusion-dependent hemo-lytic anemia. The severity of anemia may worsen during infec-tions, oxidant exposure any other physiological stress [2-4].

Enzymopathies pose a diagnostic challenge and patients may undergo repeated unsuccessful investigations over the years. Some clues include the presence of normocytic/macrocytic anemia with signs of hemolysis like indirect hyperbilirubinemia and reticulocytosis, along with a history of episodic/repeated blood transfusion for anemia. The diagnosis of a RBC enzymopathy is mainly based on exclusion; a negative DCT, a normal OFT, no specific RBC morphological abnormalities, and no evidence for abnormal hemoglobin [5]. Timely targeted NGS would help in the confirmation of diagnosis [2].

Treatment remains mainly supportive. Splenectomy is indicated in severe cases. Restoration of normal enzyme levels following bone marrow transplantation has been occasionally reported [5]. A novel treatment including enzyme activator is under development and this might provide a new option for the severe phenotype [6].

MEENA SIVASANKARAN,[1*] VAMSI KRISHNA REDDY,[2]
VIMAL KUMAR,[1] DEENADAYALAN MUNIRATHNAM[1]
From Departments of [1]Pediatric Haemato-Oncology and Bone Marrow Transplantation, and [2]Pediatrics, Kanchi Kamakoti CHILD Trust Hopsital, Numgambakkam, Chennai, Tamil Nadu.
**meenasankaran04@gmail.com*

REFERENCES

1. Koralkova P, Van Solinge WW, van Wijk R. Rare hereditary red blood cell enzymopathies associated with hemolytic anemia–pathophysiology, clinical aspects, and laboratory diagnosis. Int J Lab Hematol. 2014;36:388-97.
2. Jamwal M, Aggarwal A, Das A, et al. Next-generation sequencing unravels homozygous mutation in glucose-6-phosphate isomerase, GPIc. 1040G> A (p. Arg347His) causing hemolysis in an Indian infant. Clinica Chimica Acta. 2017;468:81-4.
3. Abrusci P, Chiarelli LR, Galizzi A, et al. Erythrocyte adenylate kinase deficiency: characterization of recombinant mutant forms and relationship with nonspherocytic hemolytic anemia. Exp Hematol. 2007;35:1182-9.
4. Grace RF, Bianchi P, van Beers EJ, et al. Clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Deficiency Natural History Study. Blood. 2018;131:2183-92.
5. Tanphaichitr VS, Suvatte V, Issaragrisil S, et al. Successful bone marrow transplantation in a child with red blood cell pyruvate kinase deficiency. Bone marrow transplantation. 2000;26:689-90.
6. Grace RF, Glader B. Red blood cell enzyme disorders. Pediatr Clin North Am. 2018;65:579-95.

Anakinra in Refractory Multisystem Inflammatory Syndrome in Children (MIS-C)

A small proportion of children can develop a hyper-inflammatory condition 2 to 4 weeks following an infection or exposure to SARS-CoV2 virus termed interchangeably as multisystem inflammatory syndrome in children (MISC) or Pediatric Inflammatory Multisystem Syndrome temporally associated with SARS-CoV-2 virus (PIMS-TS) [1]. The patho-genesis of this novel condition remains elusive and treatment protocols are predominantly empirical. Intravenous immuno-globulin (IVIG) alone or with corticosteroids are the suggested first line agents [2,3]. In those with refractory disease (defined by the presence of persistent fever and/or significant end-organ involvement despite initial immunomodulation), second line treatment options include IL-1, IL-6, and tumor necrosis factor (TNF) blockers [2,3]. The PIMS-TS arm of the RECOVERY trial is currently evaluating tocilizumab and anakinra for refractory disease [5]. The experience with use of anakinra in India is sparse due to non-availability of the drug. We report our experience with the use of anakinra in two children with refractory MISC.

Case 1: A 11-year-old boy was referred for fever, abdominal pain and diarrhea of 5 days duration. At presentation he was hypotensive (BP 70/40 mm Hg) with bilateral non purulent conjunctival suffusion and an erythematous maculopapular rash over his trunk. Investigations revealed lymphopenia (total white blood cell count (WBC) 4,350/iL, lymphocytes 4%), elevated inflammatory markers (CRP 170 mg/L, ESR 72 mm/hour, LDH 359 U/L, ferritin 1,200ng/mL, d-Dimer 12,500ng/mL), hyponatremia (130mEq/L) and increased NT-proBNP levels (>20,000pg/mL). Serology was positive for IgG SARs-CoV-2 antibodies (Chemiluminescence, titer 74.9 AU/mL). An echocardiogram showed decreased left ventricular function (LVEF, 40%). A diagnosis of MIS-C was considered, and he was given IVIG (2 g/kg) with intravenous methylprednisolone (IVMP) (2 mg/kg). Noradrenaline infusion (0.15 µg/kg/min) for hypotension and empirical antibiotics were commenced simultaneously. The dose of methylprednisolone was increased (10 mg/kg, once daily for three days), and adrenaline infusion (0.15 µg/kg/min) was started for persistent hypotension. He