

Inherited hemolytic anemias in children

Şefika Akyol¹, Akif Tahiroğlu², Ekrem Ünal^{1,3,4}

¹Erciyes University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology Oncology & Hematopoietic Stem Cell Transplant Unit, Kayseri, Türkiye

²İstanbul University, Cerrahpaşa, Faculty of Medicine, İstanbul, Türkiye

³Erciyes University, Betül Ziya Eren Genome and Stem Cell Center, Department of Molecular Biology & Genetic, Kayseri, Türkiye

⁴Erciyes University, Health Science Institution, Department of Blood Banking and Transfusion Medicine, Kayseri, Türkiye

ABSTRACT

Inherited hemolytic anemia is one of the most commonly seen anemias encountered in the pediatric age, especially in Türkiye, where consanguineous marriages are common. Inherited hemolytic anemias mainly include hemoglobinopathies, erythrocyte membrane defects, and enzyme defects. Hemolytic anemias have a wide etiology and clinical spectrum with acquired and hereditary causes in childhood. Always a careful self and family history review and synthesis of physical examination and laboratory findings are vital for differential diagnosis. Therefore, physicians should be competent in prevention methods and early diagnosis markers.

Keywords: Children, hemolytic anemia, sickle cell disease, thalassemia, inherited hemolytic anemia

INTRODUCTION

Anemia is a global health problem, affecting 42% of children less than 5 years of age worldwide (1). As it is well known, anemia is a result that can be caused by different etiologies. However, the pathogenetic mechanisms can be defined in three subtypes essentially, which are decreased production of red blood cells (RBCs), increased loss of RBCs, and premature destruction of RBCs. These three mechanisms can overlap (1-3).

Hemolysis is defined as the destruction of red cells and the release of intracellular components. The life span of the red blood cells (RBC), which is normally 100-120 days, is shortened in hemolytic anemias. The destruction of RBC can occur in both extravascular and intravascular areas (4,5). Extravascular hemolysis takes place in macrophages of the reticuloendothelial system. Clinically, it is difficult to distinguish between these two areas of hemolysis. However there are long lists of markers indicating specific types of hemolysis, the most important diagnostic tool is always the assessment of the child with a whole family history, complaints, and physical and laboratory findings on admission. Besides the major markers can be defined as the presence of hemoglobinuria and hemosiderinuria and the absence of haptoglobin are the major markers for intravascular hemolysis (4-6).

Erythropoiesis increases in the presence of hemolysis as a response. The results of increased erythropoiesis are listed in Table 1. The reticulocyte count generally exceeds 2% besides the absolute reticulocyte count higher than 100,000/ μ L. In

case of chronic hemolysis, erythropoietic marrow becomes hyperplastic, and the myeloid/erythroid ratio, which is normally 3/1, changes in favor of the erythroid series (7,8).

Table 1. Manifestations of increased erythropoiesis

1. Reticulocytosis: generally high as 10-20%.
2. Increased MCV owing to reticulocytosis
3. Increased RDW
4. Normoblasts in blood smear
5. Erythroid hyperplasia in bone marrow with increased erythroid /myeloid ratio and expansion of marrow space causing phenotypical changes like the prominence of frontal bones and broadened cheekbones, widened intratracheal places in skull radiographs and biconcave vertebrae images in X-ray.

MCV: mean corpuscular volume, RDW: red blood cell distribution width

Hemolytic anemias of childhood can be divided into two major groups. The first one is intrinsic (inherited) anemias which are composed of hemoglobinopathies, erythrocyte membrane defects, and enzyme defects. The second group is extrinsic hemolytic anemias, which typically arise from acquired disorders. In this group of diseases, hemolysis generally results from immunological, chemical, or physical damage to RBCs. This group consists of autoimmune hemolytic anemias, hypersplenism, thrombotic microangiopathies, and drug exposure (4,5). In the current review, the specific mechanisms of inherited hemolytic anemias will be addressed. Besides a general diagnosis and treatment approach will be mentioned.

HEMOGLOBINOPATHIES

Hemoglobinopathies will be elucidated into two major groups which are sickle cell disease and thalassemias (9).

Sickle Cell Disease

Sickle cell disease (SCD) arises with the formation of hemoglobin S (HbS), as a result of a point mutation that changes the amino acid glutamic acid to valine in the sixth position of the β -globin chain. The inheritance pattern of SCD is autosomal codominant. Also, coinheritance of the other beta globin mutations can develop. Hence, sickle cell disease comprises the genetic spectrum of homozygous HbS mutation, sickle-beta thalassemia, hemoglobin SC disease, and others. Approximately 300,000 children with SCD are born every year worldwide (4,10).

HbS forms sickle cell polymers under deoxygenated conditions, also HbS can not maintain the biconcave shape of the RBC, therefore capillary occlusion develops. Sickled RBC undergoes hemolysis in the intravascular and extravascular areas. Major clinical findings are related to hemolytic anemia and vaso-occlusion due to sickled erythrocytes. Hemolytic anemia can be mild to severe, worsening with infections, fever, dehydration, etc. Vasoocclusion can lead to acute and chronic pain episodes called painful crises. In addition to the painful crisis, tissue ischemia or infarct can develop. Major organs and systems can be affected by vaso-occlusion, such as the brain, lungs, kidneys, and spleen (11,12). Acute and silent infarcts are the typical clinical presentation of central nervous system involvement (13). Acute chest syndrome and vaso-occlusion in the renal arterial system can develop. Functional hyposplenism occurs in early life owing to splenic infarcts and this results in an increased risk of infection (10-14).

In laboratory evaluation, anemia with reticulocytosis is typical, as it is in most hemolytic anemias. Mean corpuscular volume can be both normal or microcytic depending on the genotype. In the presence of the SS genotype, MCV is normal, however, if concomitant α -thalassemia, $S\beta$ -thalassemia, or SC genotypes exist MCV is microcytic. Platelet count is generally increased, also neutrophilia is common. A blood smear reveals sickle cells, increased polychromasia, nucleated red cells, and target cells. Besides Howell-Jolly bodies can be observed in blood smears, indicating hyposplenism. In infants and others with high percentages of HbF, sickle cells may not take place in blood smear. Despite the high burden of inflammation, patients with SCD frequently have low erythrocyte sedimentation rates, because sickle cells fail to form rouleaux (12-15).

Diagnosis of SCD can be determined in utero with the mutation analysis of DNA from the chorionic villus biopsy or from the fetal fibroblasts obtained by amniocentesis. During the newborn period, hemoglobin electrophoresis is a useful test, after that the diagnosis should be finalized with DNA-based mutation analysis (9,10).

The treatment approach of SCD generally targets acute and chronic complications. However, it should be underlined that the prevention of complications is as precious as treatment. Specific management of these complications is out of the scope of this review.

Hydroxyurea therapy is standard-of-care SCD, representing an essential component of patient management, and has emerged as the primary disease-modifying therapy for SCD. The accumulated body of evidence over 30 years demonstrates that hydroxyurea is a safe and effective therapy for SCD. the

exact mechanisms by which hydroxyurea induces HbF and ameliorates the pathophysiology of SCD remain incompletely understood. The primary benefits of hydroxyurea for SCD relate to its ability to increase HbF level, which inhibits intracellular HbS polymerization and prevents the sickling process within erythrocytes (12-15).

Prophylactic antibiotics and proper vaccination take part in these preventive treatments. In terms of prophylactic antibiotic treatment, every child with SCD should receive oral penicillin prophylaxis with a dose of 125 mg twice daily (BID) under 3 years old and 250 mg BID for 3 years and older. Penicillin prophylaxis should start by 3-4 months of age, recommended to be continued at least at the age of 5 years old. Routine childhood vaccination, including conjugate H. influenza and Hepatitis B, should be administered to all children with SCD. Also, the 23 valent pneumococcal vaccine (PPV-23) should be applied at 2 years of age with a booster 5 years later. In addition, the conjugate 13 valent pneumococcal vaccine (PCV-13) should be administered routinely according to the schedule. Meningococcal vaccination is recommended in SCD patients as well. Influenza virus vaccination should be administered each fall, yearly. Finally, early diagnosis and treatment of infections are essential in these patients (10,14,16).

In addition to these prophylactic managements, transfusion is also frequently used in acute chest syndrome, acute strokes, and specific conditions mentioned in the treatment guidelines (16,17). Iron chelation is one of the important managements in transfusion-dependent patients (18). There are new drugs in the therapy of SCD, mentioned frequently in literature. One of them is crizanlizumab which is an anti-P selectin antibody, frequently used in painful crises (19). The other new molecule is voxelotor which is a targeted agent for the underlying mechanism. Voxelotor binds to the α -chain of the hemoglobin and modulates its affinity for oxygen, resulting in the inhibition of polymerization (20). Besides L-glutamine is an approved treatment modality for sickle cell disease (21).

Nevertheless, the main treatment approach is based on the management of the complications. Currently, hematopoietic stem cell transplantation is the only curative treatment for SCD. Besides experimental studies of gene therapy are ongoing (22).

Thalassemia

Thalassemia syndromes are one of the most common reasons for hemolytic anemias in different degrees. The main pathogenetic mechanism is based on ineffective hematopoiesis and increased hemolysis, due to reduced or absent production of one or more globin chains. The alpha/beta ratio of the globin chains varies, because of the disrupted production. Thalassemia syndromes are divided into two categories as α - and β -thalassemias, named according to the mutated or deleted globin genes. Thalassemia syndromes have clinical heterogeneity, which correlates with the degree of chain imbalance (23,24).

Beta thalassemia major, which is a severe form among the thalassemia syndromes, develops owing to absent or remarkably reduced production of beta-globin chains, because of a homozygous mutation. The frequency of beta thalassemia major has increased in societies where consanguineous marriage is common. Also, the causative mutations occur predominantly in children from the Mediterranean, African, Asian, and Southeast Asian regions. Newborn screening programs which can be applied to infants between 6-12 months of age, help the early recognition of the disease (9,25).

Pathogenesis of beta-thalassemia (either homozygous or compound heterozygous forms) can be explained by the intracellular precipitation of insoluble α -chains, because of the impaired alpha/beta ratio. Therefore, these RBCs are prematurely destroyed by frequent trapping in the spleen, resulting in increased but ineffective erythropoiesis. Consequently, the disease has three major pathogenetic pathways, which are hyperplastic bone marrow, increased iron absorption, and iron overload and hypersplenism (23-25).

Clinical features consist of pallor, growth retardation, hepatosplenomegaly, and jaundice. The diagnosis can be made by hemoglobin electrophoresis. In laboratory evaluation, the most common findings are composed of hypochromic microcytic anemia, mild reticulocytosis, leukopenia, and thrombocytopenia, with increased HbF and HbA2 levels. A blood smear reveals target cells, hypochromia, anisocytosis, polychromasia, and normoblasts. Also, owing to ineffective erythropoiesis, erythroid hyperplasia is present (23-26).

The specific treatment approach of beta-thalassemia depends on the disease severity. The clinical spectrum varies between the ones who do not require regular transfusions and the ones who can not survive unless receiving regular transfusions. Among the patients diagnosed with beta thalassemia major, if untreated, 80% of them will die within the first decade of life. Current management includes regular transfusions, monitoring for iron burden with magnetic resonance imaging (MRI) of the heart and liver and blood ferritin levels, iron chelation therapy, and management of specific complications (27,28). In addition to anemia, failure to thrive, growth retardation, cholelithiasis, hypersplenism, bone abnormalities owing to ineffective erythropoiesis, and pulmonary hypertension are the most commonly seen complications. Hematopoietic stem cell transplantation is the curative treatment option (29).

Alpha thalassemias are the syndromes named because of the globin chain, whose production is reduced. Decreased α -chain synthesis is present in these syndromes, with varying levels correlated with the genetic mutation. These syndromes have a milder phenotype compared with the beta thalassemia syndromes generally. Deletion of three α -globin genes results with HbH disease in which a significant reduction of α -chain synthesis is observed. However, patients in HbH disease have a mild clinical presentation, rarely needing blood transfusions. Deletion of all four α -globin genes culminates with the hydrops fetalis (30,31).

MEMBRANOPATHIES

Membrane defects constitute another group of inherited hemolytic anemias. Hereditary spherocytosis (HS), elliptocytosis, stomatocytosis, acanthocytosis, xerocytosis, and pyropoikilocytosis can be listed in this group of diseases (32). Herein, hereditary spherocytosis a typical and commonly seen type of membranopathy will be explained.

Hereditary spherocytosis has an autosomal dominant inheritance in the 75% of the cases. Thus it is important to keep in mind, 25% of the cases have no family history (33). The disease is common in Europe with an incidence of 1 in 5000 (34).

In pathogenesis, the primary defect is the instability of the erythrocyte membrane owing to dysfunction or deficiency of the red cell skeletal or membran proteins. The most frequent

mutation is observed in ankyrin protein accounting for 50-60% of HS cases. These mutations can occur both in dominant and recessive forms. The clinical course varies between the patients even within an affected family. The other protein deficiencies can be listed as, α and β spectrin mutations, protein 4.2 mutations, and band 3 mutations. Among them, α -spectrin mutations are observed in recessive forms of HS and account for less than 5% of HS however the clinical course is severe in this form. The other mutations have mild to moderate clinical courses. The main mechanism arises from the formation of a vertical defect in the erythrocyte membrane as a result of the reduction and/or loss of function of the proteins mentioned above, resulting in the deterioration of its integrity. In these erythrocytes, the surface area/volume ratio decreases, and spherocyte formation is observed. Spherocytes are sequestered in the spleen because they have reduced deformability compared to normal red cells, resulting in premature red cell destruction (4,34,35).

Clinical presentation of HS is usually with anemia and jaundice with varying degrees depending on the severity of the hemolysis and the compensatory erythrocytosis. Also, splenomegaly is common because the spherocytes are trapped in the spleen. The diagnosis is usually made before puberty, in fact, 50% of the patients are diagnosed in the newborn period (34-36).

In laboratory evaluation, usually mild to moderate hemolytic anemia is present. Due to the compensatory erythrocytosis, elevated reticulocytosis at 3-15% is observed. Mean cell corpuscular volume (MCV) is usually decreased, and mean corpuscular hemoglobin concentration (MCHC) and also red blood cell distribution width (RDW) are elevated. In the diagnostic approach, the presence of MCHC and RDW together is very specific for HS. The blood smear reveals spherocytes and microspherocytes in varying degrees. Also, an increased red cell osmotic fragility is determined, because the spherocytes lyse in higher saline concentrations compared to normal red cells. Diagnosis is usually made by evaluation of clinical and hematological findings and family history. Genetic analysis for the specific protein mutations can be performed however it is not essential for the diagnosis generally (34-37).

Folic acid supplementation is recommended in these patients with a dose of 1 mg/day. In case of erythroblastopenic crisis, usually seen due to parvovirus B19 infection, blood transfusions may be required. Splenectomy should be judged carefully, considering the age of the patient, the severity of the hemolysis, and accompanying complications. In the presence of severe disease, splenectomy should be done early, however, if possible it is recommended not to do it before 5 years of age. Also, the vaccination status of the patient should be assessed carefully both before and after the procedure (36,38).

ENZYMOPATHIES

Mature erythrocytes do not contain cell organelles such as mitochondria, ribosomes, or nuclei. RBCs have two biochemical systems to maintain their lifespan. One of them is Embden-Meyerhof anaerobic pathway, which provides energy production and the other one is the hexose monophosphate shunt, which provides reduction potential and protects RBC from possible toxins (39).

In the current review, the pyruvate kinase (PK) and glucose 6 phosphate dehydrogenase (G6PD) deficiency will be discussed.

Pyruvate Kinase Deficiency

PK deficiency is a rare but the most common enzyme deficiency of the Embden-Meyerhof pathway. The disease is inherited autosomal recessively and homozygous patients present with significant hemolysis. The pathogenetic mechanism is based on the defects in glycolysis and energy production of RBC. These defective red cells are rigid and fragile both metabolically and physically. Reticulocytes can produce ATP via oxidative pathways so they are less fragile in PK deficiency (40).

The clinical presentation varies between mild to moderate forms of hemolysis. Severe anemia can also be observed. Neonatal jaundice is usually present. Splenomegaly can also be detected. In the chronic period, due to hemolytic anemia, gallstones, and bone changes can be identified. Severe anemia can cause cardiomegaly and cardiac failure in these patients (41).

The diagnosis is not based on one characteristic test. In laboratory evaluation, PK deficiency has evidence of nonspherocytic hemolytic anemia. Erythrocyte PK activity can be measured and expected to be decreased to 5-20% of normal and also glycolytic metabolites like 2-3-bisphosphoglycerate (2,3-BPG) are increased. However, the diagnosis can be challenging, because of increased reticulocytes and recent red blood cell transfusions. In the diagnostic approach, other hemolytic anemias should be ruled out and the clinicians should have a high index of suspicion. Next-generation sequencing targeted panels and enzyme activity measurements can be used together to get an accurate diagnosis (40-42).

Treatment is consist of folic acid supplementation and red blood cell transfusions when required. Iron overload should be monitored and if necessary, chelation should be planned. Splenectomy is a treatment choice only in the presence of high red blood cell transfusion requirements. Also, clinical trials with the oral PK activator named mitapivat are ongoing (43).

Glucose-6-phosphate Dehydrogenase Deficiency

Glucose-6-phosphate dehydrogenase deficiency results in the diminished reductive energy of the red cell and causes hemolysis, especially increased in the presence of hemolytic agents. The inheritance is X-linked recessive. Point mutations are causative for the disease while deletion of the G6PD genes results in intrauterine death. The hemizygous males and homozygous females express the disease. In the presence of heterozygosity in females, the expression is variable due to the randomly silenced X chromosome. G6PD deficiency affects 5% of the population worldwide. As well in Türkiye the frequency was reported to vary between 0.5-2.9% with the highest rates of 8.2% in the Çukurova region (39,44,45).

The pathogenesis of the disease is based on both the decreased glucose metabolism of the red cell and diminished NADPH/NADP, reduced glutathione/GSSG ratios resulting in the impaired elimination of oxidants such as oxygen radicals. As a result, oxidation is observed in hemoglobin and the membrane of RBC. Therefore impaired integrity develops especially in the presence of oxidant drugs and infections (44,45).

Clinical presentation is usually bear on the episodic hemolysis periods induced by oxidants. The commonly observed drugs causing hemolysis in G6PD deficiency are listed in Table 2. Drug-induced hemolysis develops acutely however it is self-limiting. Heinz bodies (denatured hemoglobin precipitates) can be observed in blood smears, as well as blister cells (also called bite cells) which are formed by the removal of Heinz bodies by spleen macrophages. Also, fava bean can cause acute life-threatening

hemolysis, leading to acute renal failure. Blood transfusion can be required in severe forms of hemolysis. Another common presentation is prolonged neonatal jaundice (45).

Table 2. Drugs and agents associated with hemolysis in G6PD deficiency[§]

Analgesics and antipyretics	Acetanilide
Antimalarial agents	Pentaquine, Pamaquine, Primaquine, Quinocide
Flouroquinolones	Ciprofloxacin, Sulfonamides, Sulfanilamide, N-Acetylsulfonamide, Sulfapyridine, Sulfamethoxypyridazine, Salicylazosulfapyridine
Nitrofurans	Nitrofurazone, Nitrofurantoin, Furaltadone, Furazolidone
Sulfones	Thiazolsulfone (Promizole), Dapsone
Others	Naphthalene, Phenylhydrazine, Acetylphenylhydrazine, Toluidine blue, Nalidixic acid, Infections, Diabetic ketoacidosis

[§] Table 2 is adapted from Lanzkowsky P, editor. Manual of pediatric hematology and oncology. 7th ed. San Diego, CA:Elsevier Academic Press.

The diagnosis can be made by the measurement of enzyme activity. Nevertheless, in the presence of reticulocytosis during the hemolytic period, the enzyme levels can be normal. Therefore in the presence of high suspicion index, the test should be obtained again at least three months after the hemolytic episode (46,47).

The milestone of the treatment approach is the avoidance of agents leading to oxidative stress in RBC. Therefore education of both the parents and patients is essential. In the presence of severe and acute hemolysis with a Hb level less than 7 g/dL, red blood cell transfusion can be indicated (48).

CONCLUSION

Hemolytic anemias have a wide etiology and clinical spectrum with acquired and hereditary causes in childhood. Always a careful self and family history review and synthesis of physical examination and laboratory findings are vital for differential diagnosis. Hereditary hemolytic anemias draw attention as a public health problem, especially in societies where consanguineous marriages are common. Therefore, physicians should be competent in prevention methods and early diagnosis markers.

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