HSPI The Open Access Publisher

J A P C H JOURNAL OF ADVANCED PEDIATRICS AND CHILD HEALTH
ISSN 2689-9817

Case Report

Hereditary spherocytosis: review of cases and discussion of hematologic characteristics and updated diagnostic testing

Kennedy A Sabharwal[1] and Michael W Simon[2]*

[1]College of Medicine, University of Kentucky, USA
[2]Department of Pediatrics, University of Kentucky, USA

Abstract

Hereditary spherocytosis is a common inherited type of hemolytic anemia that results from abnormal morphology of erythrocytes. It has a high occurrence in North Americans and northern Europeans with a prevalence of 1/2000. There is a wide range in age and symptoms at presentation with some individuals being asymptomatic and others having severe diseases requiring blood transfusions. Based on the severity of symptoms, management may vary from simple observation to frequent blood transfusions, cholecystectomy for gallstones, and splenectomy. Timely diagnosis may be critical to minimize complications. Diagnostic tests have been available with varying degrees of accuracy. However new diagnostic tests with greater specificity and sensitivity are now available for more accurate diagnosis of Hereditary Spherocytosis in individuals of all ages including newborns. Illustrative cases are presented that show the variability in presentation, symptoms, complications, and care. Information is presented updating diagnostic testing that allows earlier diagnosis of children with hereditary spherocytosis. Additionally, the hematologic findings suspicious and consistent for this diagnosis are presented, serving as a guide when testing should be initiated.

More Information

***Address for Correspondence:** Michael W Simon, MD, PhD, Department of Pediatrics, University of Kentucky, USA, Email: simonmd@mwsimonmd.com

Submitted: February 20, 2023
Approved: March 10, 2023
Published: March 13, 2023

How to cite this article: Sabharwal KA, Simon MW. Hereditary spherocytosis: review of cases and discussion of hematologic characteristics and updated diagnostic testing. J Adv Pediatr Child Health. 2023; 6: 007-013.

DOI: 10.29328/journal.japch.1001054

Keywords: Hereditary spherocytosis; Hyperbilirubinemia; Anemia; Splenomegaly; Phototherapy; Osmotic fragility test

Introduction

Hereditary Spherocytosis is the most common inherited anemia in individuals of North American, northern Europe, and Japanese descent [1]. It is a disorder of erythrocyte morphology with their shape being more rigid and spherical [2]. Because of this morphological change, they are sequestered and destroyed in the spleen contributing to hemolysis and anemia [3]. They have a short half-life of 15 to 30 days. It is predominately inherited as an autosomal dominant trait with 75% of cases having this inheritance pattern [4]. 25% may be inherited as an autosomal recessive and de novo accounting for sporadic cases of this disorder [4].

The specific defect involves proteins under the lipid layer involving mutations in the Spectrin, Ankyrin, band 3, and protein 4.2 proteins altering membrane stability and shape [1]. There are specific patterns of protein deficiency contributing to this membrane stability: 1 Partial Spectrin and protein 4.2 deficiency. 2. Partial Ankyrin and Spectrin deficiency. 3. Partial Spectrin deficiency. 4. Marked deficiency of Spectrin. 5 Partial bands 3 deficiency. 6. Partial protein 4.2 deficiency. Autosomal recessive cases are more likely to be caused by a defect in either alpha Spectrin or protein 4.2 [1]. De novo mutations are common for Ankyrin 1 and beta Spectrin defects.

Hereditary Spherocytosis may be diagnosed with the onset of symptoms which may range from the newborn up through the third or fourth decade of life [5]. Most often it is diagnosed in those between three and seven years of age. Those with Hereditary Spherocytosis may remain asymptomatic with their bone marrow keeping up with the degree of erythrocyte loss and require observation. Others may have severe symptoms requiring frequent transfusions, cholecystectomy for biliary gallstones, and splenectomy [6]. Phagocyte activity activated by hemolysis may increase the concentration of toxic free radicals further contributing to the spherocyte membrane damage.

Diagnostic testing has improved for accuracy in both specificity and sensitivity allowing for the accurate diagnosis

of Hereditary Spherocytosis at any age for an individual with consistent or suspicious signs of this disorder. An individual may not be considered to have Hereditary Spherocytosis until they present with a hemolytic crisis or aplastic crisis secondary to Parvovirus 19 or influenza infections [7]. The most accurate test that may be done today is the Eosin-5-Maleimide (EMA) test which measures the deficiency of the band-3 protein and has a 90% sensitivity and > 99% specificity [8]. Illustrative cases have been presented that show the variability in presentation, symptoms, complications, care, and diagnostic testing for Hereditary Spherocytosis.

Cases

Case #1

A now 9-year-old white female was diagnosed with hereditary spherocytosis at 2 ½ years of age. She was 7 pounds and 5 ounces at birth and term by dates and size. She had an uncomplicated vaginal delivery with unremarkable resuscitation. Her newborn exam was normal. She breastfed well and at 48 hours of age was discharged home on phototherapy for a bilirubin of 15.7 (Normal (N) 0.2 - 12.0 mg/dL).

The following day she was reevaluated and noted to be markedly jaundiced. Her bilirubin was 26.7 mg/dL. She was readmitted for phototherapy. Admission labs included AST 33 (N 10-20 µ/L), ALT 15 (N 10-36 /L), and alkaline phosphatase 175 (N < 455 µ/L). The white blood cell (WBC) count was 12.9 (N 6.0 – 17.5 µ/L), RBC 3.38 (N 4.80 - 7.10 µ/L), hematocrit 29.7 (N 42% - 66%), MCV 88 (N 80 - 100 FL), MHC 32 (N 27 - 31 PG), MCHC 36.4 (N 32 - 36 g/dL), RDW 19.6 (M 11.3% - 14.5 %), and platelets 822,000 (N 140 - 190 k/µL). The Coombs test was negative. She received 3 days of phototherapy and was discharged with a bilirubin of 13.5 mg/dL. Her jaundice was presumed to be secondary to breast milk.

At 2 ½ years of age, she was noted to have icterus with a concurrent viral illness. The examination was remarkable for icterus plus palpable spleen 3-4 cm below the left costal margin her total bilirubin was 4.6 mg/dL, all indirect (N 0.1 - 1.1 mg/dL). The WBC was 7.32 (N 6.00 - 17.00 k/µL), RBC 3.08 (N 3.90 - 5.30 m/µL), hematocrit 26.4 (N 34.0% - 40.0%), MCV 85.7 (N 75.0 - 87.0 FL), MHC 30.5 (N 24.0 – 30.0 PG), MCHC 35.6 (N 31.0 - 37.0 g/dL), RDW 19.7 (N 11.3% - 14.5%) and platelets 325,000 (N 150 – 450 k/µL). Morphology showed spherocytes. Osmotic fragility was 0.71 (N 0.41% - 0.58%) consistent with hereditary spherocytosis. She was started on folic acid 1.0 mg/ day).

Annual physicals have shown a palpable spleen 3 cm - 4 cm below the left costal margin with a baseline total bilirubin of 2.0 – 3.0 mg/dL, direct bilirubin 0.1 – 0.3 mg/ dL, and hematocrit 28% – 30%. At 5 years of age, ultrasound showed gallstones and the spleen 4 cm - 5 cm below the left costal margin. Total bilirubin was 7.5 mg/dL and direct bilirubin 0.4 mg/dL. The hematocrit was 28%. At 6 years of age, she had a total splenectomy and cholecystectomy. Her jaundice has completely resolved, and she continues to do well at routine follow-ups. She has received pneumococcal and meningococcal vaccinations.

Case #2

A now 8-year-old white female was diagnosed with hereditary spherocytosis at 9 months of age. The mother has hereditary spherocytosis and had a splenectomy as a child. The infant was the 8 lb 6 oz 36-week product delivered by cesarean section for fetal distress. Resuscitation was unremarkable. The newborn examination was normal, and she was breastfed. The infant developed jaundice and had a serum bilirubin level of 22.1 (N 0.2 – 12.0 mg/dL), all indirect (N 0.6 – 10.5 mg/dL). At 72 hours of age the hematocrit was 45.6 (N 35.0% – 45.0%), RBC 4.76 (N 4.00 – 5.20 m/µL), MCV 75.8 (N 70.0 – 86.0 FL), MCH 34.5 (N 23 – 31.0 PG), MCHC 36.0 (N 30.0 – 36.0 g/dL), RDW 17.2 (N 11.3% – 14.5%), and platelet count 508,000 (N 150 – 450 k/µL). Coombs test was negative and liver enzymes were normal.

She was started on phototherapy and continued breastfeeding. After 48 hours of therapy, the bilirubin level was 17.7 mg/dL. Breastfeeding was stopped and after another 24 hours of treatment, the bilirubin level dropped to a total serum level of 14.2 (N 0.2 – 12.0 mg/dL), direct 0.4 (N 0.0 – 0.6 mg/dL), and indirect 13.8 (N 0.6 – 10.5 mg/dL). She was discharged home with no additional measures. She was reevaluated at 8 days for jaundice. The total bilirubin was 18.6 mg/dL. Home phototherapy began and decreased the serum bilirubin to 13.7 (N 0.2 – 12.0 mg/dL), indirect 13.5 (N 0.6 – 10.5 mg/dL), and direct 0.2 (N 0.0 – 0.6 mg/dL) after 72 hours. Phototherapy was discontinued no rebound was noted.

The infant did well until 9 months of age. She developed a viral illness with pallor. Laboratory studies included a hematocrit 27.6 (N 33.0% – 39.0%), RBC 3.53 (N 3.70 – 5.30 m/µL), MCV 78.2 (N 70.0 – 86.0 FL), MCH 28.3 (N 23.0 – 31.0 PG), MCHC 36.2 (N 30.0 – 36.0 g/dL), RDW 15.5 (N 11.3% - 14.5%), platelets 727,000 (N 150 – 450 k/µL), and reticulocyte count 6.99 (N 0.5% – 1.5%). Hematology consultation confirmed hereditary spherocytosis with an osmotic fragility test result of 0.61 (N 0.46% – 0.58%).

The patient had an aplastic crisis at 6 years of age from a parvovirus infection, but no treatment was needed. Otherwise, she has done well in the interim with no abdominal pain, icterus, or pallor. Her hematocrit remains between 28.6% – 35.6% (N 33.0% – 40.0%), RBC 3.5 – 4.3 (N 3.70 – 5.30 m/dL), MCV 78 – 88 (N 70.0 – 86.0 FL), MCH 29.1 – 32.1 (N 23.0 – 31.0 PG), MCHC 36.9 – 37.8 (N 30.0 – 36.0 g/µL), RDW 13.9 – 17.4 (N 11.3% – 14.5%), and reticulocyte count 5.5 – 8.8 (N 0.5% – 1.5%).

Her spleen remains palpable 2 cm below the left costal

margin. Ultrasound at 6 years of age showed no gallstones and a spleen 10.5 cm in size. She remains asymptomatic and is not a candidate for splenectomy or cholecystectomy.

Case #3

A now 5-year-old male was diagnosed with hereditary spherocytosis at 2 months of age. He is the younger sibling of case #2. Mother has hereditary spherocytosis and had a splenectomy as a child. He was 9 lb and 11 oz and term by dates and size. He had a spontaneous vaginal delivery with unremarkable resuscitation. His newborn examination was normal. He breastfed well. He was noted to have jaundice with maximum total bilirubin of 15.8 (N 0.6 – 10.5 mg/dL), all indirect, at 3 days of age. No treatment was required.

Due to a family history of hereditary spherocytosis, he was evaluated by a hematologist at 2 months of age. His RBC count was 4.19 (N 3.2 – 5.4 m/μL), hematocrit 25.6 (N 28.0% – 42.0%), MCV 80 (N 70 - 90 FL), MCHC 36.4 (N 33.2 – 35.3 g/dL), RDW 13.8 (N 12% – 16%), reticulocyte count 5.3 (N 0.5% – 2.5%) and absolute reticulocyte count 224 (N 20 – 130 k/μL). The total bilirubin was 1.5 (N 0.2 – 12.0 mg/dL) and direct 0.3 (N 0.0 – 0.6 mg/dL). The osmotic fragility test was 72.0 (N 0.0% – 47.8%).

At 3 years of age, he was admitted for an aplastic crisis secondary to parvovirus infection and treated with a single packed red blood cell transfusion. He continues to grow and develop well. He has had no episodes of jaundice, icterus, or abdominal pain. He has had no splenomegaly or symptoms of gallbladder disease. His RBC indices have remained normal, except for his RDW ranging between 15.9 – 18.7 (N 12% – 16%). His hematocrit ranges from 30% – 32% (N 33% – 42%). The reticulocyte count varies between 5.0 – 7.8 (N 0.05% – 2.5%) with the absolute reticulocyte count 250 – 315 (N 20 – 130 k/μL). He has been evaluated but is not currently a candidate for splenectomy or cholecystectomy.

Case #4

A now 20-year-old was diagnosed with hereditary spherocytosis at 6 months of age. He was the 8 lb 8 oz product 43 weeks by dates and size delivered by spontaneous vaginal delivery. He has no family history of hereditary spherocytosis and one normal sibling. He had an unremarkable neonatal course and was breastfed during his first two months of life.

At 2 months of age, he had a viral illness. He was noted to have pallor and spleen palpable 4 cm below the left costal margin. His hematocrit was 17% (N 35% – 45%) and his reticulocyte count was 17.2% (N 0.5% – 1.5%). His other laboratory values were normal and diagnostic evaluation unremarkable. He received a transfusion with packed red blood cells.

Reevaluation at 4 months of age showed his spleen to be 5 cm below the left costal margin. The hematocrit was 34% (N 34% – 45%) and the reticulocyte count was 3.8% (N 0.5% – 1.5%). Two weeks later, the hematocrit was 23.8% (N 34% – 45%). At 5 months of age, he had icterus. His spleen was found to be 3.5 cm below the left costal margin. The hematocrit was 19.7 (N 34% – 45%), RBC 2.9 (N 4.0 – 5.2 m/μL), MCV 82.4 (N 77 – 95 FL), MCHC 33.5 (N 25 – 33 g/dL), MCH 27.6 (N 31 – 37 PG), RDW 22.8 (N 11.3% – 14.5%) and reticulocyte count 18.0 (N 0.5% – 1.5%). Spherocytes were noted on the peripheral smear. The report from pathology was that the erythrocyte morphology was consistent with viral hemolytic anemia; spherocytes noted were not as uniform as seen with hereditary spherocytosis cases.

The infant was examined by a hematologist at 6 months of age. He had icterus and spleen palpable 5 cm below the left costal margin. The hematocrit was 16.7 (N 35% – 45%), RBC 2.11 (N 4.0 – 5.2 m/μL), MCV 79 (N 77 – 95 FL), MCH 27.5 (N 31 – 37 PG), MCHC 34.7 (N 25 – 33 g/dL), RDW 17.9 (N 11.3% – 14.5%), and reticulocyte count 14.2 (N 0.5% – 1.5%). Total bilirubin was 1.4 (N 0.2 – 1.0 mg/dL) and osmotic fragility test 0.62 (N 0.40 – 0.58%). He received a transfusion with packed red blood cells.

Between 6 months and 8 years of age, he continued to have splenomegaly and icterus. His lab values ranged between hematocrit 26.9 – 27.9 (N 34% – 45%), MCV 79 – 83 (N 77 – 95 FL), MHC 28.4 – 29.7 (N 31 – 37 PG), MCHC 35.7 – 36.1 (N 25 – 33 g/dL), RDW 17.9 – 22.8 (N 11.3% – 14.5%), and reticulocyte count 16.9 – 17.6 (N 0.5% – 1.5%) and total bilirubin 2.9 – 3.5 (N 0.2 – 1.2 mg/dL). The osmotic fragility test was 0.68% (N 0.40% – 0.58%) at 7 years of age. Ultrasound of the gallbladder showed no gallstones and splenomegaly with the spleen measuring 13.2 x 8.2 cm.

Splenectomy was performed on the patient at 8 years of age. His labs have normalized, hematocrit ranging between 42.1 – 47.8 (N 34% – 45%), RBC 5.25 – 5.31 (N 4.0 – 5.2 m/μL), MCV 79.3 – 91 (N 77 – 95 FL), MCH 28.6 – 32.2 (N 31 – 37 PG), MCHC 35.2 – 36.3 (N 25 – 33 g/dL) and RDW 12.8 – 13.3 (N 11.3% -14.5%). He has received pneumococcal and meningococcal vaccinations. He continues to do well at routine follow-ups.

At the time of this report, the individuals reported have become adults and have been lost to follow-up.

Discussion

Overview of hereditary spherocytosis

Hereditary spherocytosis is one of the most common inherited causes of hemolytic anemia in children. It is the result of a mutation in one of five genes that codes for proteins important for the erythrocyte membrane cytoskeleton of the phospholipid bilayer producing their spherical shape. The mutated proteins involved are Spectrin, Ankyrin, band 3 and band 4.2. The most frequent protein deficiencies are band 3 and Spectrin.

Early diagnosis will help to monitor an individual with hereditary spherocytosis reducing the risk of complications later in life. Any family history of hereditary spherocytosis warrants an examination for this disorder. Spherocytosis predisposes to hemolysis. As a result, it may even present in utero as hydrops fetalis or cause neonates to have prolonged or exaggerated jaundice with elevated indirect bilirubin [9]. Jaundice due to severe hemolysis is uncommon after the neonatal period. Neonates rarely have splenomegaly. Due to polycythemia at birth, they may not have anemia until the second or third week of life or even more delayed with an elevated reticulocyte count [10]. Infants may require transfusions during their first year of life. After this time, it is unusual to need transfusions unless hereditary spherocytosis is severe [1]. Older children and adults may present with hemolytic anemia, elevated indirect bilirubin, splenomegaly, and cholelithiasis or may have an aplastic crisis with infection. Bacterial and viral infections especially Parvovirus 19 or influenza may cause transient aplastic anemia [11]. Mononucleosis may cause splenomegaly and contribute to erythrocyte pooling and hemolysis due to the enlarged spleen [12]. The different types of protein structure variation may contribute to the timing and severity of symptoms for hereditary spherocytosis.

Classification of hereditary spherocytosis

Hereditary spherocytosis may be classified based on markers of hemolysis (bilirubin and reticulocyte count) and degree of anemia [13-15].

- Mild hereditary spherocytosis (20% - 30% of cases): hemoglobin 11 - 15 g/dl (gram/deciliter); reticulocyte count 3-6% (N 0.5% – 1.5%); bilirubin 1 - 2 mg/dl (milligram/deciliter); few spherocytes.

- Moderate hereditary spherocytosis (60% - 75% of cases): hemoglobin 8 - 12 g/dl; reticulocyte count > 6%; bilirubin >2 mg/dl; 5% - 20% spherocytes.

- Severe hereditary spherocytosis (5% of cases): hemoglobin 6 - 8 g/dl; reticulocyte count > 10%; bilirubin > 2 mg/dl; 20% - 30% spherocytes.

Laboratory testing for hereditary spherocytosis

Routine laboratory testing through nonspecific screening often shows elevated (MCHC) mean corpuscular hemoglobin concentration, elevated bilirubin level and reticulocytes, and the presence of spherocytes. With hemolysis, there may be low haptoglobin and high lactate dehydrogenase (LDH) [16]. The Coombs test (direct anti-globulin test, DAT) is negative in hereditary spherocytosis [17]. The first clinical clues would include spherocytes as seen on a blood smear and an elevated MCHC level. The complete blood count (CBC) is the first step in evaluating for hereditary spherocytosis. Individuals known to have hereditary spherocytosis almost always are found to have an abnormally high MCHC [18,19]. Levels greater than 35 g/dL are consistently observed in children with hereditary spherocytosis. Newborns and children through two years of age have an average MCHC of 30.3 g/dl [20,21]. Those two years of age and older have an average MCHC of 30.4 g/dl [20]. An MCHC greater than 35.5 g/dl is indicative of hereditary spherocytosis [20,21]. This was similarly observed in three of our four patients who presented with an MCHC greater than or equal to 36.0 g/dl range 36.0 – 36.4 G/DL. All 4 of our patients had at least one MCHC greater than or equal to 35.5 g/dl at some point observed in their lifespan. Using the MCHC as a marker for hereditary spherocytosis it has a sensitivity of 82% and specificity of 94% to 98% [22]. A review from the author's primary care practice, 93 patients were selected at random ranging between 10 to 18 years of age, and had a total of 200 complete blood count tests performed. 20 of these CBCs had an MCHC greater than or equal to 35.5 G/DL. Of note, for this population, there were no additional cases of hereditary spherocytosis diagnosed. This shows that an elevated MCHC alone is not diagnostic for hereditary spherocytosis but may be suspicious as a marker of disease.

Other indices may be used to help diagnose hereditary spherocytosis. In hereditary spherocytosis the red blood cell distribution width (RDW) is elevated [23,24]. They have a smaller red blood cell volume (MCV) than normal erythrocytes [14]. The mean sphere corpuscular volume (MSCV) is also low in hereditary spherocytosis [15,16,25,26]. Typically, they have a high reticulocyte count to compensate for hemolysis [27]. With the CBC, a blood smear with manual inspection of the blood smear would be the next step in looking for spherocytes. Up to one-third of neonates with hereditary spherocytosis may not have prominent spherocytes [28]. Unfortunately, the presence of spherocytes on a blood smear during the first few weeks of life is also not diagnostic of hereditary spherocytosis [28]. It may be easier and more accurate to assess the erythrocyte morphology at six months of age and older [1].

Presentation of hereditary spherocytosis in the newborn

Newborns may have an elevated total serum bilirubin from several causes including the normal physiological transition for new babies as well as Rh or ABO blood incompatibility, polycythemia from maternal-fetal transfusion, delayed cord clamping, placental insufficiency, maternal diabetes, or hemolytic factors related to enzymatic deficiencies, hemoglobinopathies or variations in erythrocyte morphology. Children with metabolic deficiencies for B12, folate, or iron may not be able to maintain sufficient erythrocyte production to compensate for hemolysis.

It has been proposed that children with a positive family history of hereditary spherocytosis with spherocytes noted on a blood smear, elevated MCHC, and increased erythrocyte count do not require any additional testing. As many as 75% of those with hereditary spherocytosis will have a positive

family history [29]. However, if the diagnosis is equivocal confirmatory testing is recommended. There are different specific confirmatory testing that may be done [14,30].

Confirmatory testing

The most common confirmatory test has been the osmotic fragility test. For this test, erythrocytes are incubated in a hypotonic buffered salt solution in various osmolarities and the level of hemoglobin from hemolysis is measured. It is not specific for hereditary spherocytosis but will capture spherocytes from any cause. The spherocytes are more likely to hemolyze producing a higher test result. This test has a low sensitivity (60% -66%) and specificity of 81% with a high false negative rate with as many as 10% to 25% having a normal test result, especially in neonates [13,31]. For this test to be accurate, at least 2% of the erythrocytes must be spherocytes. For this reason, mild cases may be missed.

The osmotic gradient ektacytometry (OGE) method also measures osmotic fragility due to erythrocyte deformability [32]. It measures the deformity of the entire erythrocyte population. It cannot distinguish between hereditary spherocytosis and autoimmune hemolytic disease [33]. It is not routinely available in most clinical laboratory settings. The glycerol lysis test (GLT) and the acidified glycerol lysis test (AGLT) are similar but have modifications to the osmotic fragility test. The glycerol lysis test adds glycerol and acidified glycerol lysis test adds glycerol plus sodium phosphate to lower the pH of the testing solution. Both use a hypotonic buffered salt solution. The level of hemoglobin from hemolyzed erythrocytes is measured. The GLT has a 61% sensitivity and the AGLT has a 93% - 95% sensitivity [34-36]. A modification of the glycerol lysis test is called the pink test. It measures the amount of hemolysis in a glycerol solution and the pH of 6.6. The test sample may be collected by a finger prick or heel puncture of a newborn is called “the direct pink test”. It is incubated directly in the glycerol solution and requires a small amount of blood. It has a 91% sensitivity. The GLT, AGLT, and pink tests may not detect mild cases of hereditary spherocytosis [37-40]. The Cryohemolysis test (CHT) suspends erythrocytes in a hypertonic solution and is briefly heated to 37°C then cooled to 4°C. It likewise measures the degree of hemolysis in comparison between spherocytes and normal cells. Overall, it is easy to perform but has limited availability and may be unreliable [31,39,41-44].

The Eosin-5-maleimide (EMA) test is a flow cytometry test that uses an Eosin-based fluorescent dye that binds to erythrocyte membrane proteins, especially band-3. In hereditary spherocytosis, the deficiency in these proteins produces less fluorescence compared to normal individuals as measured by flow cytometry. It is the most specific test for hereditary spherocytosis [40,41,45-47]. Its accuracy is not age-dependent and will be accurate even in neonates [29]. It has a sensitivity ranging from 93% to 96% and a specificity ranging from 93% to 99% [31]. False negatives may occur in mild cases of hereditary spherocytosis. Because it is simple, highly reproducible, cost-effective, and faster but accurate, it may now be the diagnostic test of choice for confirming the diagnosis of hereditary spherocytosis.

No single test reliably identifies all individuals with hereditary spherocytosis. However, the accuracy may be improved by combining two different tests. Combining the EMA binding with a GLT had a sensitivity of 100%. When the EMA test is combined with the cryo-hemolysis test, the sensitivity is 93%. Combining the EMA binding and pink test produces a sensitivity of 99%. If EMA binding is combined with the osmotic fragility test either incubated or fresh there is a sensitivity of 95% [30]. Combining the osmotic fragility test with the AGLT has a sensitivity of 97% [31]. When combined, if both the EMA and AGLT tests are negative, the individual does not have hereditary spherocytosis.

Another higher-level testing may be done but is generally limited to a research laboratory. They require a greater sample volume, more preparation, laborious, and time-consuming. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) would be a specialty test evaluating the cytoskeleton membrane proteins and identifying the deficient proteins. It is the only test that will differentiate hereditary spherocytosis from congenital diserythropoietic anemia type II [31]. Alternatively molecular diagnostics, DNA analysis, and sequencing may be done. It is expensive and usually the last option for those suspected of having hereditary spherocytosis but who have had negative testing.

Conclusion

Hereditary Spherocytosis is a common inherited form of anemia that may go unnoticed and undiagnosed for years in childhood. The range of symptoms may present as asymptomatic to children requiring transfusion and splenectomy. The information presented in this report updates diagnostic testing to allow for earlier diagnosis of children with Hereditary Spherocytosis. The EMA test is the single most specific diagnostic test and may be used in neonates. Although no single test reliably diagnoses all children with HS, dual testing with EMA binding and GLT provides great sensitivity and the combination of EMA binding with an AGLT test shows great specificity. Another test like SDS-PAGE will identify the specifically deficient proteins in patients with Hereditary Spherocytosis.

References

1. Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. Lancet. 2008 Oct 18;372(9647):1411-26. doi: 10.1016/S0140-6736(08)61588-3. PMID: 18940465.

2. Bajracharya BL, Giri A, Baral MR. Hereditary spherocytosis. Kathmandu Univ Med J (KUMJ). 2004 Apr-Jun;2(2):145-8. PMID: 15821383.

3. Le CH. The Prevalence of Anemia and Moderate-Severe Anemia in the US Population (NHANES 2003-2012). PLoS One. 2016 Nov

15;11(11):e0166635. doi: 10.1371/journal.pone.0166635. PMID: 27846276; PMCID: PMC5112924.

4. Kliegman R. Nelson Textbook of Pediatrics. Elsevier. 2020; (21): 2531.
5. Friedman EW, Williams JC, Van Hook L. Hereditary spherocytosis in the elderly. Am J Med. 1988 Mar;84(3 Pt 1):513-6. doi: 10.1016/0002-9343(88)90275-6. PMID: 3348251.
6. Cecil R, editor. Goldman's Cecil Medicine. 24th ed. Philadelphia.
7. Cohen H, Walker H, Delhanty JD, Lucas SB, Huehns ER. Congenital spherocytosis, B19 parvovirus infection and inherited interstitial deletion of the short arm of chromosome 8. Br J Haematol. 1991 Jun;78(2):251-7. doi: 10.1111/j.1365-2141.1991.tb04425.x. PMID: 1648374.
8. Arora RD, Dass J, Maydeo S, Arya V, Radhakrishnan N, Sachdeva A, Kotwal J, Bhargava M. Flow cytometric osmotic fragility test and eosin-5'-maleimide dye-binding tests are better than conventional osmotic fragility tests for the diagnosis of hereditary spherocytosis. Int J Lab Hematol. 2018 Jun;40(3):335-342. doi: 10.1111/ijlh.12794. Epub 2018 Mar 24. PMID: 29573337.
9. Gallagher PG, Weed SA, Tse WT, Benoit L, Morrow JS, Marchesi SL, Mohandas N, Forget BG. Recurrent fatal hydrops fetalis associated with a nucleotide substitution in the erythrocyte beta-spectrin gene. J Clin Invest. 1995 Mar;95(3):1174-82. doi: 10.1172/JCI117766. PMID: 7883966; PMCID: PMC441455.
10. Delhommeau F, Cynober T, Schischmanoff PO, Rohrlich P, Delaunay J, Mohandas N, Tchernia G. Natural history of hereditary spherocytosis during the first year of life. Blood. 2000 Jan 15;95(2):393-7. PMID: 10627440.
11. Elbadry MI, Khaled SAA, Ahmed NM, Abudeif A, Abdelkareem RM, Ezeldin M, Tawfeek A. Acute human parvovirus B19 infection triggers immune-mediated transient bone marrow failure syndrome, extreme direct hyperbilirubinaemia and acute hepatitis in patients with hereditary haemolytic anaemias: multicentre prospective pathophysiological study. Br J Haematol. 2021 May;193(4):827-840. doi: 10.1111/bjh.17484. Epub 2021 Apr 25. PMID: 33899219.
12. Gehlbach SH, Cooper BA. Haemolytic anaemia in infectious mononucleosis due to inapparent congenital spherocytosis. Scand J Haematol. 1970;7(2):141-4. doi: 10.1111/j.1600-0609.1970.tb01879.x. PMID: 5450688.
13. Wu Y, Liao L, Lin F. The diagnostic protocol for hereditary spherocytosis-2021 update. J Clin Lab Anal. 2021 Dec;35(12):e24034. doi: 10.1002/jcla.24034. Epub 2021 Oct 24. PMID: 34689357; PMCID: PMC8649336.
14. Manciu S, Matei E, Trandafir B. Hereditary Spherocytosis - Diagnosis, Surgical Treatment and Outcomes. A Literature Review. Chirurgia (Bucur). 2017 Mar-Apr;112(2):110-116. doi: 10.21614/chirurgia.112.2.110. PMID: 28463670.
15. Bolton-Maggs PH, Langer JC, Iolascon A, Tittensor P, King MJ; General Haematology Task Force of the British Committee for Standards in Haematology. Guidelines for the diagnosis and management of hereditary spherocytosis--2011 update. Br J Haematol. 2012 Jan;156(1):37-49. doi: 10.1111/j.1365-2141.2011.08921.x. Epub 2011 Nov 5. PMID: 22055020.
16. Chavez-Bueno S, Beasley JA, Goldbeck JM, Bright BC, Morton DJ, Whitby PW, Stull TL. 'Haptoglobin concentrations in preterm and term newborns'. J Perinatol. 2011 Jul;31(7):500-3. doi: 10.1038/jp.2010.197. Epub 2011 Jan 20. PMID: 21252963.
17. Bolton-Maggs PH, Stevens RF, Dodd NJ, Lamont G, Tittensor P, King MJ; General Haematology Task Force of the British Committee for Standards in Haematology. Guidelines for the diagnosis and management of hereditary spherocytosis. Br J Haematol. 2004 Aug;126(4):455-74. doi: 10.1111/j.1365-2141.2004.05052.x. PMID: 15287938.
18. Christensen RD, Henry E. Hereditary spherocytosis in neonates with hyperbilirubinemia. Pediatrics. 2010 Jan;125(1):120-5. doi: 10.1542/peds.2009-0864. Epub 2009 Nov 30. PMID: 19948573.
19. Michaels LA, Cohen AR, Zhao H, Raphael RI, Manno CS. Screening for hereditary spherocytosis by use of automated erythrocyte indexes. J Pediatr. 1997 Jun;130(6):957-60. doi: 10.1016/s0022-3476(97)70283-x. PMID: 9202619.
20. Oski F: Pallor. In: Kaye R, Oski F, Barness L eds. Core Textbook of Pediatrics, 3rd ed. Philadelphia: Lippincott; 1989: 62
21. Akar N, Gökçe H. Red blood cell indexes in patients in patients with hereditary spherocytosis and beta-thalassemia combination. Pediatr Hematol Oncol. 2002 Dec;19(8):569-73. doi: 10.1080/08880010290097431. PMID: 12487832.
22. Liao L, Xu Y, Wei H, Qiu Y, Chen W, Huang J, Tao Y, Deng X, Deng Z, Tao H, Lin F. Blood cell parameters for screening and diagnosis of hereditary spherocytosis. J Clin Lab Anal. 2019 May;33(4):e22844. doi: 10.1002/jcla.22844. Epub 2019 Apr 3. PMID: 30945356; PMCID: PMC6528600.
23. Steward SC, Chauvenet AR, O'Suoji C. Hereditary spherocytosis: Consequences of delayed diagnosis. SAGE Open Med. 2014 Aug 16;2:2050312114547093. doi: 10.1177/2050312114547093. PMID: 26770738; PMCID: PMC4607200.
24. Mullier F, Lainey E, Fenneteau O, Da Costa L, Schillinger F, Bailly N, Cornet Y, Chatelain C, Dogne JM, Chatelain B. Additional erythrocytic and reticulocytic parameters helpful for diagnosis of hereditary spherocytosis: results of a multicentre study. Ann Hematol. 2011 Jul;90(7):759-68. doi: 10.1007/s00277-010-1138-3. Epub 2010 Dec 22. PMID: 21181161.
25. Broséus J, Visomblain B, Guy J, Maynadié M, Girodon F. Evaluation of mean sphered corpuscular volume for predicting hereditary spherocytosis. Int J Lab Hematol. 2010 Oct;32(5):519-23. doi: 10.1111/j.1751-553X.2009.01216.x. Epub 2010 Feb 2. PMID: 20136849.
26. Lazarova E, Pradier O, Cotton F, Gulbis B. Automated reticulocyte parameters for hereditary spherocytosis screening. Ann Hematol. 2014 Nov;93(11):1809-18. doi: 10.1007/s00277-014-2127-8. Epub 2014 Jun 10. PMID: 24912770.
27. King MJ, Garçon L, Hoyer JD, Iolascon A, Picard V, Stewart G, Bianchi P, Lee SH, Zanella A; International Council for Standardization in Haematology. ICSH guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders. Int J Lab Hematol. 2015 Jun;37(3):304-25. doi: 10.1111/ijlh.12335. Epub 2015 Mar 18. PMID: 25790109.
28. Christensen RD, Yaish HM, Lemons RS. Neonatal hemolytic jaundice: morphologic features of erythrocytes that will help you diagnose the underlying condition. Neonatology. 2014;105(4):243-9. doi: 10.1159/000357378. Epub 2014 Feb 8. PMID: 24526179.
29. Chari PS, Prasad S. Flow Cytometric Eosin-5'-Maleimide Test is a Sensitive Screen for Hereditary Spherocytosis. Indian J Hematol Blood Transfus. 2018 Jul;34(3):491-494. doi: 10.1007/s12288-017-0907-8. Epub 2017 Dec 6. PMID: 30127559; PMCID: PMC6081319.
30. Zaidi AU, Buck S, Gadgeel M, Herrera-Martinez M, Mohan A, Johnson K, Bagla S, Johnson RM, Ravindranath Y. Clinical Diagnosis of Red Cell Membrane Disorders: Comparison of Osmotic Gradient Ektacytometry and Eosin Maleimide (EMA) Fluorescence Test for Red Cell Band 3 (AE1, SLC4A1) Content for Clinical Diagnosis. Front Physiol. 2020 Jun 19;11:636. doi: 10.3389/fphys.2020.00636. PMID: 32636758; PMCID: PMC7318840.
31. Bianchi P, Fermo E, Vercellati C, Marcello AP, Porretti L, Cortelezzi A, Barcellini W, Zanella A. Diagnostic power of laboratory tests for hereditary spherocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics. Haematologica. 2012 Apr;97(4):516-23. doi: 10.3324/haematol.2011.052845. Epub 2011 Nov 4. PMID: 22058213; PMCID: PMC3347664.
32. Llaudet-Planas E, Vives-Corrons JL, Rizzuto V, Gómez-Ramírez P, Sevilla Navarro J, Coll Sibina MT, García-Bernal M, Ruiz Llobet A, Badell I, Velasco-Puyó P, Dapena JL, Mañú-Pereira MM. Osmotic gradient ektacytometry: A valuable screening test for hereditary

spherocytosis and other red blood cell membrane disorders. Int J Lab Hematol. 2018 Feb;40(1):94-102. doi: 10.1111/ijlh.12746. Epub 2017 Oct 10. PMID: 29024480.

33. Lazarova E, Gulbis B, Oirschot BV, van Wijk R. Next-generation osmotic gradient ektacytometry for the diagnosis of hereditary spherocytosis: interlaboratory method validation and experience. Clin Chem Lab Med. 2017 Mar 1;55(3):394-402. doi: 10.1515/cclm-2016-0290. PMID: 27559691.

34. Eber SW, Pekrun A, Neufeldt A, Schröter W. Prevalence of increased osmotic fragility of erythrocytes in German blood donors: screening using a modified glycerol lysis test. Ann Hematol. 1992 Feb;64(2):88-92. doi: 10.1007/BF01715351. PMID: 1554800.

35. Mittler U, Radig K, Kluba U, Aumann V, Röppnack R. Erfahrungen mit dem Glyzerol-Lyse-Test im sauren Milieu (AGLT) bei der Diagnostik der hereditären Sphärozytose [Experience with the glycerol lysis test in acid medium in diagnosis of hereditary spherocytosis]. Kinderarztl Prax. 1993 Aug;61(6):219-22. German. PMID: 8411849.

36. Hoffmann JJ, Swaak-Lammers N, Breed WP, Strengers JL. Diagnostic utility of the pre-incubated acidified glycerol lysis test in haemolytic and non-haemolytic anaemias. Eur J Haematol. 1991 Nov;47(5):367-70. doi: 10.1111/j.1600-0609.1991.tb01862.x. PMID: 1761123.

37. Parpart AK, Lorenz PB, Parpart ER, Gregg JR, Chase AM. THE OSMOTIC RESISTANCE (FRAGILITY) OF HUMAN RED CELLS. J Clin Invest. 1947 Jul;26(4):636-40. doi: 10.1172/JCI101847. PMID: 16695456; PMCID: PMC439273.

38. Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. Lancet. 2008 Oct 18;372(9647):1411-26. doi: 10.1016/S0140-6736(08)61588-3. PMID: 18940465.

39. Crisp RL, Solari L, Vota D, García E, Miguez G, Chamorro ME, Schvartzman GA, Alfonso G, Gammella D, Caldarola S, Riccheri C, Vittori D, Venegas B, Nesse A, Donato H. A prospective study to assess the predictive value for hereditary spherocytosis using five laboratory tests (cryohemolysis test, eosin-5'-maleimide flow cytometry, osmotic fragility test, autohemolysis test, and SDS-PAGE) on 50 hereditary spherocytosis families in Argentina. Ann Hematol. 2011 Jun;90(6):625-34. doi: 10.1007/s00277-010-1112-0. Epub 2010 Nov 16. PMID: 21080168.

40. Korones D, Pearson HA. Normal erythrocyte osmotic fragility in hereditary spherocytosis. J Pediatr. 1989 Feb;114(2):264-6. doi: 10.1016/s0022-3476(89)80794-2. PMID: 2915286.

41. Park SH, Park CJ, Lee BR, Cho YU, Jang S, Kim N, Koh KN, Im HJ, Seo JJ, Park ES, Lee JW, Yoo KH, Jung HL. Comparison study of the eosin-5'-maleimide binding test, flow cytometric osmotic fragility test, and cryohemolysis test in the diagnosis of hereditary spherocytosis. Am J Clin Pathol. 2014 Oct;142(4):474-84. doi: 10.1309/AJCPO7V4OGXLIIPP. PMID: 25239414.

42. Emilse LAM, Cecilia H, María TM, Eugenia MM, Alicia IB, Lazarte SS. Cryohemolysis, erythrocyte osmotic fragility, and supplementary hematimetric indices in the diagnosis of hereditary spherocytosis. Blood Res. 2018 Mar;53(1):10-17. doi: 10.5045/br.2018.53.1.10. Epub 2018 Mar 27. PMID: 29662857; PMCID: PMC5898988.

43. Mariani M, Barcellini W, Vercellati C, Marcello AP, Fermo E, Pedotti P, Boschetti C, Zanella A. Clinical and hematologic features of 300 patients affected by hereditary spherocytosis grouped according to the type of the membrane protein defect. Haematologica. 2008 Sep;93(9):1310-7. doi: 10.3324/haematol.12546. Epub 2008 Jul 18. PMID: 18641031.

44. Romero RR, Poo JL, Robles JA, Uriostegui A, Vargas F, Majluf-Cruz A. Usefulness of cryohemolysis test in the diagnosis of hereditary spherocytosis. Arch Med Res. 1997 Summer;28(2):247-51. PMID: 9204617.

45. Christensen RD, Agarwal AM, Nussenzveig RH, Heikal N, Liew MA, Yaish HM. Evaluating eosin-5-maleimide binding as a diagnostic test for hereditary spherocytosis in newborn infants. J Perinatol. 2015 May;35(5):357-61. doi: 10.1038/jp.2014.202. Epub 2014 Nov 6. PMID: 25357094.

46. Joshi P, Aggarwal A, Jamwal M, Sachdeva MU, Bansal D, Malhotra P, Sharma P, Das R. A comparative evaluation of Eosin-5'-maleimide flow cytometry reveals a high diagnostic efficacy for hereditary spherocytosis. Int J Lab Hematol. 2016 Oct;38(5):520-6. doi: 10.1111/ijlh.12533. Epub 2016 Jun 24. PMID: 27339613.

47. King MJ, Telfer P, MacKinnon H, Langabeer L, McMahon C, Darbyshire P, Dhermy D. Using the eosin-5-maleimide binding test in the differential diagnosis of hereditary spherocytosis and hereditary pyropoikilocytosis. Cytometry B Clin Cytom. 2008 Jul;74(4):244-50. doi: 10.1002/cyto.b.20413. PMID: 18454487.