An infant with chronic hemolytic anemia

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Case

A 7-month old male patient was referred to our clinic because of mild anemia and icteric sclerae. The patient had a history of intensive phototherapy which was applied for three days because of neonatal jaundice which developed on the second day of life. In the follow-up performed in the external center where the delivery took place, moderate reticulocytosis, increased indirect bilirubin and decreased haptoglobulin which suggested chronic hemolysis were found. In the familial history, it was learned that the parents were third degree relatives. A history of chronic jaundice and anemia was present in the patient's aunt's daughter who was one year old. On physical examination, the conjunctivae were slightly pale, the sclerae were icteric; the spleen was nonpalpable and percussion of the Traube's space was found to be normal. The neuromotor development of the patient was appropriate for age. The necessary laboratory tests were performed.

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Diagnosis : Glucose phosphate isomerase deficiency

Detailed biochemical tests including complete blood count, peripheral blood smear, indirect Coomb test, direct-indirect bilirubin and lactate dehydrogenase (LDH) levels were performed in the patient. Mild hypochomia, anisocytosis and poikilocytosis were observed on peripheral blood smear. There was no erythrocyte deformity which would suggest membrane disease. Hemogram revealed the following: Hb: 9 g/dL, HCT: 27%, MCV: 81 fL, MCHC: 32 g/dL, reticulocyte count: 3%. Direct and indirect Coombs tests were found to be negative, indirect bilirubin value: 3.5 mg/dL (normal: <2 mg/dL), LDH: 800 IU/L (normal: <600 IU/L). Other biochemical variables were found to be normal. The blood type of the mother and children was O Rh (+). Serum haptoglobulin value was found to be low (8 mg/dL) (normal: 16-200 mg/dL). The complete blood counts and peripheral blood smears of the parents were found to be normal. No abnormal band was found in hemoglobin electrophoresis of the patient (Hb A: 98%, Hb A2: 2%). In the incubated osmotic fragility test, the resistance of the erthyrocytes was normal. Glucose 6 phosphate dehydrogenase (G6PDH) and pyruvate kinase (PK) enzyme levels were within the normal limits. Physical examination revealed no pathological finding and no finding suggesting hypersplenism. There was no history of drug usage. The level of glucose phosphate isomerase (GPI) which is observed more rarely in the population was measured in the patient who had values which supported chronic hemolysis. The GPI level was found to be low (32%) (normal: 85-100%).

Discussion

Anemia is a very common health problem in children. Especially in communities in developing countries, 80% of the people present to a hospital because of anemia at least for one time during their childhood (1). In hemolytic anemia, erythrocytes which have a normal life span of 100-120 days are eliminated from the circulation in a shorter time (2). Hemolytic anemia may be observed in a healthy individual because of immune-mediated mechanisms, drugs and infections. In hemolytic anemies caused by erythrocyte enzyme defects, membrane defects or hemoglobinopathies, the course is chronic. Exceptions of this include G6PD and gluthatione synthetase deficiency which are characterized with acute hemolytic attacks. The classification of hemolytic anemies is shown in Table 1.

In hemolytic anemia, the bone marrow which is healthy synthesizes more erythrocytes than normal as a response to increased erythropoetin; the reticulocyte count increases above 2%. When the hemolytic process becomes chronic, the myeloid/erythroid ratio which is 3/1 increases in favour of the erythroid cells. In conditions where there is deep anemia including major thalassemia, enlargement in some bones including mainly the facial bones and bones in the hands is observed as a result of extension of increased activity in the bone marrow to the medullary area. The hemolytic process is demonstrated directly by determining the life span of the erythrocytes (with radioactive sodium chromate; Cr-51) or indirectly by measurement the metabolites of hemolysis (increased indirect bilirubin and LDH, decreased haptoglobulin). Even if the indirect bilirubin values are higher than normal, marked jaundice may not be observed. If there is no accompanying hepatic disease, the total bilirubin level rarely increases above 5 mg/dL. Urinary and fecal urobilinogen level is increased because of destruction of bilirubin. Increased ex-

Table 1. Causes of hemolytic anemia in children

Causes related with erythrocytes

- Hemoglobinopathiesr
  - Thalassemias, alpha thalassemia, beta thalassemia (major/intermedia)
  - Sickle cell anemia
  - Abnormal hemoglobins; HbC, Hb Lepore, Hb D Los Angeles, Hb O Arab, Hb Constant Spring, HB E
- Erythrocyte membrane diseases
  - Hereditary spherocytosis
  - Hereditary eliptocytosis
  - Hereditary piropiokilocytosis
  - Hereditary stomatositosis
  - Hereditary xerocytosis
- Erythrocyte enzyme deficiencies
  - Hexose monophosphate pathway deficiencies; G6PD deficiency
  - Other gluthatione metabolism disorders; gluthatione synthetase and gluthatione peroxidase deficiency
  - Glycolytic pathway deficiencies; PK, GPI, PPK, TPI, PGK, aldolase deficiency
  - Erythrocyte nucleotide metabolism disorder; P-5'-N deficiency
- Paroxysmal nocturnal hemoglobinuria

Extra-erythrocyte causes

- Hemolytic anemias which develop with antibodies
  - Warm antibodies
  - Cold antibodies
  - Paroxysmal cold hemoglobinuria
  - Incompatible blood transfusion
  - Neonatal hemolytic disease
- Hemolysis related with drugs, chemical substances
- Hemolysis caused by infections
- Hypersplenism

G6PD: glucose 6phosphate dehydrogenase; PK: piruvate kinase; GPI: glucose phosphate isomerase; PPK: phoosphofructokinase; TPI: triose phosphate isomerase; PGK: phosphoglycerate kinase; P-5-N: Pyrimidine 5'-nucleotidase

Table 2. Properties of chronic hemolytic anemies

Disease	Inheritance, pathology	Clinical picture	Peripheral blood smear, Laboratory findings
Beta-thalassemia major	Autosomal recessive Beta-chain synthesis defect	The clinal findings appear after the 6th month; deep anemia, Hb:<7 g/dL, dependent on regular transfusion Hepatosplenomegaly, modification in facial bones	Anisocytosis, polychromasia, target cell, normoblast Hb electrophoresis; HbF >50%, HbA2:< 4%
Beta-thalassemia intermedia	Autosomal recessive Beta-chain synthesis defect	The diagnosis is made after the age of 2 years Moderate anemia, Hb: 7-10 g/dL Clinical variable: there may be no need for transfusion- transfusion may be needed	Anisocytosis, polychromasia, target cell, normoblast Hb electrophoresis; Hb F: 10-50%, Hb A2:> 4%
Alpha-thalassemia (HbH disease)	Autosomal recessive alpha-chain synthesis defect	Findings appear in the neonatal period Moderate anemia, Hb: 7-10 g/dL Hepatosplenomegaly, gallstone, leg ulcer	Hypochromia, microcytosis, normoblast HbH inclusion (with supravital staining) Hb electrophoresis: 0.8%-40 HbH (beta4)
Sickle cell anemia	Autosomal co-dominant hemoglobinopathy	Clinical findings appear in the 4th month at the earliest Chronic anemia progressing with acute attacks; Vasoocclusive*, aplastic and splenic sequestration crisis	Sickle cell, target cell Hb electrophoresis; Hb S and Hb F are found
Hereditary spherocytosis	Autosomal dominant (75%) Non-dominant inheritance (%25) erythrocyte membrane defect	Different clinical course; mild-moderate-deep anemia (Hb<6-12 g/dL) Dependent on transfusion-asymptomatic course Neonatal jaundice, rarely deep anemia Splenomegaly	Spherocyte MCHC ↑ OF ↑ (incubated test)
Hereditary eliptocytosis/HPP	Autosomal dominant Erythrocyte membrane defect	Clinical course is variable; In utero mortality, hydrops fetalis, chronic transfusion-asymptomatic course Anemia; mild-moderate-deep	Elyptocyte (>5%), spherocyte, poikilocytosis
Hereditary stomatocytosis	Autosomal dominant erythrocyte membrane defect	Clinical course is variable; chronic hemolytic anemia-asymptomatric course	Stomatocyte (5-50%), macrocytosis
Hereditary xerocytosis	Autosomal dominant erythrocyte membrane defect	Clinical course is variable; Perinatal ascites, hydrops fetalis, pseudohyperkalemia-asymptomatic course	Xerocytosis MCV and MCH ↑ OF ↓
G6PD deficiency	X-linked recessive erythrocyte membrane defect	Neonatal jaundice Acute hemolytic anemia** Chronic anemia†	Bite cells, Heinz body in erythrocytes (blister cell with supravital staining) Erythrocyte G6PD activity ↓
PK deficiency	Autosomal recessive erythrocyte membrane defect	Clinical picture varies with enzyme activity; In utero/neonatal anemia, kernicterus-anemis in adulthood	Spiculated pyknocytes Erythrocyte PK activity ↓
GPI deficiency	Autosomal recessive erythrocyte membrane defect	Clinical picture varies with enzyme activity; Hydrops fetalis/neonatal anemia, kernicterus-anemis in adulthood Neurodegenerative disease, granulocyte disorders	Erythrocyte GPI activity ↓ (below 85% of the normal level)
Hexokinase deficiency	Autosomal recessive erythrocyte membrane defect	Clinical picture is variable; Neonatal jaundice, need for regular erythrocyte transfusion-asymptomatic course	Target cell, echinocyte Erythrocyte HK activity ↓
PPK deficiency	Autosomal recessive erythrocyte membrane defect	If only erythrocyte enzyme is deficient, anemia in adolescence If muscle enzyme is also deficient, blood lactate ↓ after exercise Myopathy, neonatal mortality	Basophilic stippling Erythrocyte PPK activity ↓
TPI deficiency	Autosomal dominant erythrocyte enzyme deficiency	The degree of anemia is variable Sever neuromuscular disease accompanies (progrssive)	Macrocytosis, polychromasia Erythrocyte TPI activity ↓
PGK deficiency	Autosomal dominant erythrocyte enzyme deficiency	The degree of anemia is variable Neurological finding may accompany	Erythrocyte PGK activity ↓

Table 2. Continued

Disease	Inheritance, pathology	Clinical picture	Peripheral blood smear, Laboratory findings
Aldolase deficiency	Autosomal recessive erythrocyte membrane defect	Clinical picture is variable; Severe chronic hemolytic anemia-asymptomatric course Neurological finding may accompany	Erythrocyte aldolase activity ↓
P-5'-N deficiency	Autosomal recessive erythrocyte membrane defect	The degree of anemia is variable Growth retardation may be observed	Basophilic stippling Erythrocyte P-5'-N activity ↓
Gluthatione synthetase deficiency	Autosomal recessive erythrocyte membrane defect	Hemolysis in oxidative stres Intermittant neutropenia, metabolic acidosis	Eryhtrocyte gluthatione synthase level ↓

G6PD: glucose 6 phosphate dehydrogenase; GPI: glucose phosphate; KH: hexokinase; HPP: hereditary propoikilocytosis, OF: osmotic fragility; PGK: phosphoglycerate kinase; TPI: triose phosphate isomerase

*Vaso-occlusive crisis; painful crisis, acute chest syndrome, acute abdominal pain, acute central nervous system event and priapism

**Acute hemolytic anemia; triggered by drugs, naphtaline and infection, causes Hb to drop to 2.5 g/dL and intravascular hemolysis

†If the enzyme level is below 1%, life-long dependence to transfusion.

cretion of bilirubin pigments due to chronic hemolysis causes to formation of pigmented gallstones in the childhood. In any condition where hemolysis occurs, hemoglobin released into the plasma irreversibly binds to serum haptoglobulin. This complex which is formed is eliminated from the circulation in the liver. Since the level of haptoglobulin is limited, it is measured to be low during hemolysis (<20 mg/dL) or is not found in the serum. However, a low haptoglobulin level is not always a reliable indicator for hemolysis; haptoglobulin level is found to be low without hemolysis in haptoglobulin deficiency which is observed with a rate of 30% in the population in Western Africa. Since haptoglobulin is an acute phase reactant, it is found to be increased in cases of infection and inflammatory conditions. It should be kept in mind that the haptaglobulin level can not be measured accurately in infants younger than 2-3 months. In patients with chronic hemolytic anemia, the spleen is slightly-moderately enlarged excluding sickle cell anemia in which the spleen is enlarged to a great degree. Although leg ulcer may be observed specifically in patients with hereditary spherocytosis and sickle cell anemia, it may also be observed in other hemolytic diseases with a much lower frequency.

In the differential dignosis of chronic hemolytic anemia, the time and severity of the symptoms are important. Patients present with jaundice in the neonatal period in hereditary spherocytosis, alpha thalassemia and G6PD and PK deficiency, whereas the first findings generally appear after the 4-6th month in beta thalassemia. While patients with thalassemia major need chronic blood transfusion, some of the patients with hereditary xerocytosis and stomatocytosis may be asymptomatic for a life time. While glutathione synthetase and G6PD enzyme deficiency generally progress with acute attacks, hemolysis is chronic in all the remaining anemias. Presence of additional signs including mainly neurological findings is directive especially in the diagnosis of erythrocyte membrane defects (Table 2). The main laboratory tests include evaluation of a well-prepared peripheral blood smear by an experienced hematologist in addition to complete blood count. The primary tests include osmotic fragility test, hemoglobin electrophoresis, G6PD and PK enzyme levels. The levels of enzymes deficiencies of which occur more rarely are measured, if the previous tests are inconclusive. Familial history is valuable in the diagnosis of hemolytic anemies. Since many erythrocyte enzyme deficiencies and thalassemias are autosomal recessive diseases, patients should be interrogated in terms of consanguineous marriage and history in the siblings. Osmotic fragility and hemoglobin electrophoresis tests do not give an accurate result after erythrocyte suspension transfusion or in the neonatal period. In such cases, performance of these tests in the mother and father are helpful in the diagnosis. Table 2 shows the characteristics of chronic hemolytic anemias.

Glucose phosphate isomerase is an enzyme which reversibly catalyses transformation of glucose 6 phosphate and fructose 6 phosphate to each other which is the second step of anaerobic glycolysis in the Embden Meyerhof pathway. Therefore, it is an essential part of carbonhydrate metabolism. It is observed considerably rarely, although it is the second most common glycolytic enzyme disease following piruvate kinase deficiency (3). About 50 cases have been reported in the literature, though its frequency has not been clarified exactly (4). Glucose 6 phosphate which is increased because of deficiency of the enzyme inhibits hexokinase enzyme which transforms glucose to glucose 6 phosphate with negative feedback and thus the glycolysis and hexose monophosphate pathway is supressed. The synthesis of gluthatione, 2-3 diphosphoglycerate and adenozine triphosphate decreases. In this condition, the erythrocyte membrane loses its stabilization and a chronic non-spherocytic anemia develops. In case of exposure to oxidants including viral infections or antibiotherapy, acute hemolytic attacks may be observed as a result of decreased synthesis of antioxidant substances (5). Hydrops fetalis may even be observed, when the disease has a severe course (6). Glucose phosphate isomerase plays a role as a neurotrophic factor for spinal and sensory neurons in its monomeric form in addition to its enzymatic activity. Thus neu-

rodegenerative diseases associated with muscle weakness, ataxia, dysarthria and mental retardation may be observed in addition to hemolytic anemia in its deficiency (7). In addition, granulocyte disorders and splenomegaly have been reported in some patients (8). Since it is an autosomal recessive diseases, women and men are affected equally. There is no clinical finding in carriers. Clinical findings are observed in homozygous or combined heterozygous individuals. The diagnosis of the disease is made by excluding other anemias causing to hemolysis and with an enzyme activity lower than 85% of the normal value. The parents of our patient were third-degree relatives. The fact that the same disease was observed in the aunt's daugther who was born from a consanguineous marriage (third degree relatives) was directive for us.

In this article, a patient with chronic hemolytic anemia caused by GPI deficiency which starts in the neonatal period and which is observed rarely was presented and chronic hemolytic anemies were reviewed hereby. The diagnosis of glucose phosphate isomerase deficiency should be kept in mind in the follow-up of a patient with hemolytic anemia. In this article, it was emphasized that the cause may be GPI deficiency in chronic hemolytic anemia.

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