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CASE REPORT
ΕΝΔΙΑΦΕΡΟΥΣΑ ΠΕΡΙΠΤΩΣΗ

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Hereditary spherocytosis in a young male
Report of an unusual case

**T. Kanellopoulou,
F.N. Kontopidou,
S.P. Dourakis**

Second Department of Internal Medicine, "Hippokration" General Hospital of Athens, Medical School, University of Athens, Athens, Greece

Hereditary spherocytosis (HS) is the most frequent form of red blood cell membrane disorder and the most common cause of chronic hereditary hemolytic anemia. The etiology of the disease is a deficiency in the membrane proteins which results in instability of the cytoskeleton. The case is presented of a 32-year-old man who was admitted with pain in the right hypochondrium, jaundice and hyperpigmentation of the urine. HS due to protein-3 deficiency was diagnosed and the clinical syndrome at admission was attributed to bile duct obstruction. The laboratory methods which determine the diagnosis of HS are discussed and reference is made to the therapeutic management of the patients. The search for HS should not be omitted in the investigation of chronic hemolysis, because of the variety of clinical manifestations of the disease, which may remain asymptomatic and undetected even in old age.

Κληρονομική σφαιροκυττάρωση σε νεαρό ενήλικα. Περιγραφή ασυνήθους περιστατικού

Περίληψη στο τέλος του άρθρου

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Hereditary spherocytosis (HS) is the most common hereditary disorder of the red cell membrane and the most common cause of chronic hemolytic anemia in Caucasians, and it is characterized by marked heterogeneity. The estimated prevalence ranges from 1:2,000 to 1:5,000.[1] The characteristic picture is that of spherical, osmotically fragile erythrocytes the shape of which is caused by defects in the membrane proteins. These erythrocytes are selectively trapped by the spleen. The molecular defect involves the genes encoding for spectrin, ankyrin, band 3 and protein 4.2. The defective protein can be detected by sodium-dodecyl-sulphate polyacrilamide gel electrophoresis (SDS-PAGE) which permits the identification of various different subsets of patients.[1–3] The major complications of HS are aplastic and megaloblastic crises, hemolytic crisis, severe neonatal hemolysis, cholecystitis and cholelithiasis.[1,4–6] This is a case report of common jaundice (hypebilirubinemia) and right hypochondrial pain in a man with spherocytosis. The diagnosis of this hereditary disease was not made until the age of 32 years.

CASE REPORT

A 32-year-old man from Albania presented with a 3-day history of jaundice, right hypochondrial pain and hyperpigmentation of urine. The main symptom which brought him to the hospital was abdominal pain. He had no family or personal history of severe illnesses, no history of neonatal jaundice, and was not taking any drugs. On examination, his general condition was not considered severe, but he was pale and moderately jaundiced. His liver and spleen were both enlarged and palpated 2 cm and 18 cm, respectively below the costal margin and the Murphy sign was positive. He had no lymphadenopathy and all other systemic examinations were normal. The laboratory data were as follows: WBC 6.6×10^3 cells/μL (the morphology of white blood cells and the differential count were normal), RBC 2.88×10^6/μL, Hb 9.1 g/dL, Ht 25%, PLT 117×10^3/μL, MCV 87 fL, MCH 31.7 pg, MCHC 36.5 g/dL, RDW 24.3% (Blood Counter *Beckman Coulter LH-750*), ESR 17 mm, total-bilirubin 15.06 mg/dL, direct-bilirubin 1.71 mg/dL, LDH 401 U/L, ALT 283 IU/L, AST 409 IU/L, γGT 103 IU/L, ALP 117 IU/L, total cholesterol 65 mg/dL, HDL 26 mg/dL, LDL 18 mg/dL. The direct antiglobulin test was negative, haptoglobin <28.3 mg/dL, and Fe, ferritin, TIBC and folic acid were within normal range. The peripheral smear showed polychromasia, anisocytosis and a few spherocytes (fig. 1). Hemoglobinopathies were excluded (Hb

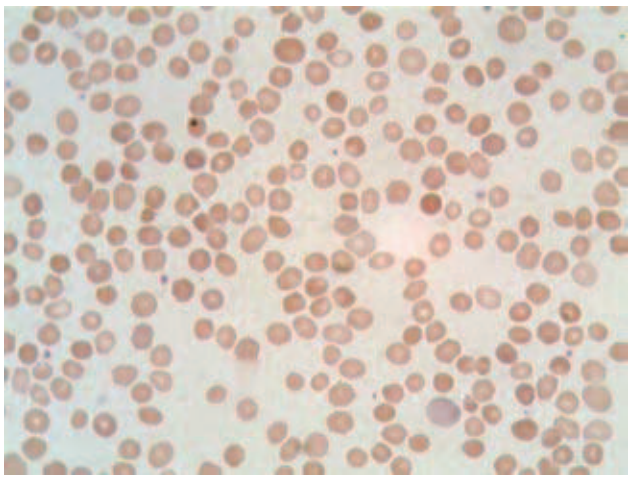

Figure 1. May-Grünwald Giemsa stain. Peripheral smear with polychromasia, anisocytosis and spherocytosis.

electrophoresis: HbA_2 2.8%, HbF 2.6%, HbS 0%) and testing for Hb-H and unstable hemoglobins was negative. The erythrocytes were assayed for the enzyme G6PD which was detectable at the level of 320 mu/10^9 RBC (normal range >118).[7] Heinz bodies were not detected. The reticulocyte count was 18% and the reticulocyte indices were: MRV 74.7 fL (normal range 100–125), MCSV 65.6% (normal range 84–104). MCSV was lower than MCV which has sensitivity of 100% and specifity of 93% in spherocytosis. Data indicated that spherocytosis might be a possible diagnosis.[8] The next test that was performed was osmotic fragility, which showed increased osmotic fragility of the erythrocytes in hypotonic solutions (index of reduced cell volume and surface area) (fig. 2). SDS-PAGE of red blood cell membrane protein, using gel acrylamide according to Laemmli's and Fairbanks' method, showed that the patient had a deficiency of band 3: Spectrin/Band 3=1.32 (normal range 1±0.02), Ankyrin/Band 3=0.24 (normal range 0.17±0.02). Quantitation of bands was expressed as ratios to band 3. Protein 4.2 was not defined.[3,4]

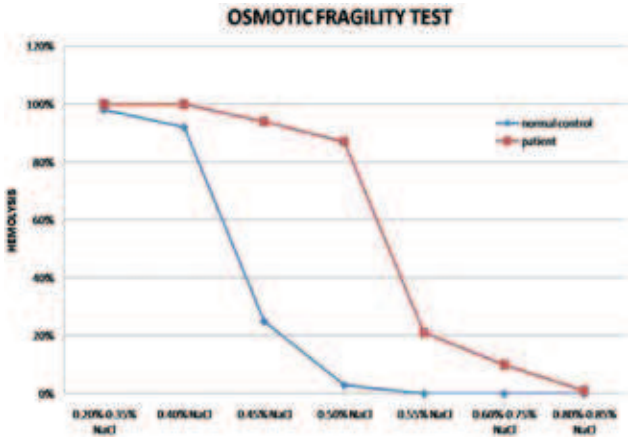

Figure 2. The osmotic fragility test with increased osmotic fragility of erythrocytes in hypotonic solutions.

Five days after admission, spontaneous improvement was observed; the jaundice and the abdominal pain improved progressively. Laboratory data showed total bilirubin 7 mg/dL, direct bilirubin 6 mg/dL. Ultrasound (US) of the abdomen demonstrated hepatomegaly (maximum diameter 19.1 cm), splenomegaly (maximum diameter 20.2 cm) and the gallbladder was full of gallstones and sludge, without dilatation of the biliary duct. Following these findings, the clinical signs and laboratory abnormalities were ascribed to a temporary obstruction of the bile duct. On discharge, the patient was vaccinated against *S. pneumoniae, H. influenzae* and *N. meningitidis,* and one month later he underwent cholecystectomy and splenectomy laparoscopically and was advised to take 1 mg folic acid per day.[3] The laboratory findings immediately before operation were: WBC 10×10^3 cells/μL, Hb 11.3 g/dL, Ht 30.7%, PLT 150×10^3/μL, total bilirubin 6.23 mg/dL, direct bilirubin 0.46 mg/dL, LDH 291 U/L, AST 22 IU/L, ALT 11 IU/L, ALP 63 IU/L, γGT 9 IU/L, total cholesterol 71 mg/dL, HDL 26 mg/dL, LDL 9 mg/dL. The peripheral blood smear showed spherocytes, microspherocytes, polychromasia and RET about 15%. After the operation, mild reactive thrombocytosis was observed. G6PD assay was repeated and was normal. He was monitored as an outpatient and clinical improvement was observed.

DISCUSSION

Hereditary spherocytosis comprises of a heterogeneous group of disorders with regard to clinical severity, protein defects and mode of inheritance. It is the commonest inherited red cell membrane disorder, although it is generally a rare disease worldwide. This disorder is associated with increased hemolysis, the degree of which depends on the interplay between an intact spleen and an intrinsic membrane protein defect. The abnormal morphology and the shorter lifespan of the red cells in hereditary spherocytosis are attributable to a deficiency or dysfunction of one of the constituents of the red cell cytoskeleton, the role of which is to maintain the shape, deformability and elasticity of the red cell. Hemolysis is primarily confined to the spleen and therefore is extravascular.[2,4]

The abnormal red cell morphology, which results in shortened cell survival, is due to a deficiency of, or a dysfunction in, spectrin, ankyrin, band 3 and or protein 4.2. Spectrin deficiency is the most common defect. A variety of mutations have been noted in genes encoding these membrane proteins. The genes responsible are localized on chromosomes 1, 2, 8, 15 and 17 for membrane proteins. Most cases of hereditary spherocytosis are heterozygous because homozygous states are lethal. In pedigrees that have a dominant defect, affected family members tend to have similar degrees of hemolysis and clinical severity. Severe hemolytic anemia is often associated with a greater reduction of the affected membrane protein(s). There

is an apparent correlation between clinical and protein phenotypes. [4,11]

The clinical severity of HS varies from a symptom-free carrier state to severe hemolysis. Mild HS can be difficult to identify because individuals may have normal levels of hemoglobin and bilirubin. Although the diagnosis of HS is often made in childhood and young adulthood, it may be diagnosed at any age.[1,4]

The peripheral blood smear shows numerous spherocytes. Larger bluish cells (polychromasia) also may be seen. The complete blood count (CBC) and reticulocyte count reveal a low hemoglobin concentration and elevated reticulocyte count. The MCHC is usually high, with levels greater than 35 g/dL. The MCV may be low, or high if there is substantial reticulocytosis. Hematology analysers using the principle of flow cytometry (i.e. dual angle laser light scattering method) produce a more accurate determination of red cell volume (MCV) and hemoglobin concentration (MCHC) than blood cell analysers using the principle of electrical aperture impedance. These automated red cell parameters can be used to predict or identify hereditary spherocytosis with a typical clinical presentation during the routine CBC without requiring additional laboratory tests (such as osmotic fragility or the EMA binding test) to confirm the diagnosis.[8] The test of osmotic fragility can be useful in establishing the diagnosis of HS. A normal osmotic fragility result does not exclude the diagnosis of HS and may occur in 10–20% of cases of HS. The test may also be normal in the presence of iron deficiency, obstructive jaundice, and in the recovery phase from an aplastic crisis when the reticulocyte count is increased. Cell dehydration occurring in the spherocytes of a patient with HS can be one of the causes of normal osmotic fragility that results in non-splenectomized patients. A positive osmotic fragility result may also be obtained in patients with hereditary elliptocytosis (HE) and hemolysis.[4] The cryohemolysis test, the osmotic gradient ektacytometry and the EMA binding test have a higher predictive value in the diagnosis of HS because there have been no reports of positive results in immune or non-membrane-associated disorders. Identification of a deficiency in a membrane protein associated with erythrocyte cytoskeleton confirms the diagnosis of HS. Quantification of membrane proteins by SDS-PAGE is, however, not necessary for the majority of cases because a definitive diagnosis can be made on the basis of red cell indices, the clinical/family history and a positive result from a screening test.[4] Co-inheritance of other hematological disorders, such as beta thalassemia trait or sickle cell disease, can lead to confusion in the diagnosis. Iron, folate or vitamin B_{12} deficiencies can mask the laboratory features. Obstructive jaundice alters the lipid composition of the red cell membrane, masking the film appearances and reducing the hemolysis.

The patient presented had moderately severe hemolysis, as was evidenced by low hemoglobin, persistent reticulocytosis, jaundice, hepatosplenomegaly, hypocholesterolemia. Hereditary spherocytosis was suggested by the following findings: Hyperdense red cells, reticulocytosis and reticulocyte indices (MCSV<MCV) and increased osmotic fragility. The direct antiglobulin test was negative. The diagnosis was confirmed by partial reduction of band 3 protein (found by SDS-PAGE of red blood cell membrane proteins).

The molecular pathology of HS is heterogeneous and total absence of band 3 has been reported in few cases. Band 3 reductions can be detected in 10–20% of patients with the benign, dominantly inherited pattern. As a result, it may be diagnosed at an older age, even after the age of 50 years.[4,5,9–11] Spectrin deficiency is more frequently diagnosed in childhood and band 3 deficiency in adulthood. The Hb is slightly lower, and spherocyte numbers and hemolysis markers higher in spectrin deficiency than in band 3 deficiency as well. In addition, splenomegaly and gallstones are more frequent in band 3 deficient patients whereas anemia, neonatal jaundice and transfusion requirement is more common in those with spectrin or ankyrin deficiency.[1,3,4]

The complication of common jaundice, which was noted in this patient on admission, was ascribed to a temporary obstruction of bile duct. Hypocholesterolemia observed was ascribed to chronic anemia with increased erythropoietic activity. The exact etiology of hypocholesterolemia in these patients is not known and the data are insufficient, but it is thought to be due to increased cholesterol requirements of the proliferating erythroid cells.

The clinical diagnosis in a typical case of HS is usually straightforward and a family history is very important, found in nearly 75% of cases.[11] A family study could not be carried out in this case since the patient's family resides in another country, but he denied a similar history in any member of the family. Thus, this case may be either recessively inherited, or sporadic as occurs in 25% of cases or a silent carrier state, as it has been suggested to occur in 1.4% of the population. It is important to ask for a family history of jaundice and or splenectomy, as families may not realize that the cause for such events involves their red blood cells.

HS in this case report was characterized as moderate and the patient underwent concurrent cholecystectomy

and splenectomy laparoscopically. The decision for this type of operation was based on the following data: Anemia (Hb <12 g/dL), reticulocytosis (RET count >10%), jaundice (total bilirubin >6 mg/dL) and complications of the disease, in this case gallstones.[3]

It is important to differentiate HS from hereditary stomatocytosis and related disorders with abnormal permeability of sodium and potassium ions. These disorders are rare; the morphology may not be typical, particularly where films are not freshly made. Such families have been labelled as "atypical" HS. Splenectomy is not effective in stomatocytosis and is associated with a high risk of thrombosis.

Most children and adults with HS have mild to moderate enlargement of the spleen. The size of the spleen alone is not an indication for splenectomy. Splenectomy is very effective in reducing hemolysis, leading to a significant prolongation of the red cell lifespan, although not necessarily to normal. The clinical manifestations and complications (anemia and gallstones) are much reduced in severe HS and abolished in milder cases by splenectomy, but at the price of an increased risk of life-threatening sepsis from encapsulated organisms, particularly *Streptococcus pneumoniae*. Patients should be selected for splenectomy on the basis of their clinical symptoms and presence of complications such as gallstones, and not simply on the basis of the diagnosis alone.[3] Recently, it has been shown that the nature of the membrane defect (whether predominantly spectrin/ankyrin, or band 3) and severity of deficiency influences the response to splenectomy.

Guidelines for splenectomy in patients with HS are not very clear and the final decision will rest on consultation between the family and the clinician, having in mind the infection risks, such as postsplenectomy sepsis, and their management. No published evidence of the optimum timing for any type of resection exists. The timing of splenectomy depends on the severity of the patient's symptoms as judged by the effects of anemia, symptoms related to cholelithiasis or the need for transfusions.[3]

The diagnosis of HS should always be considered as a cause of gallstones and splenomegaly in adult life, as there may be a considerable interval between the onset of symptoms and diagnosis.

ΠΕΡΙΛΗΨΗ

Κληρονομική σφαιροκυττάρωση σε νεαρό ενήλικα. Περιγραφή ασυνήθους περιστατικού

Θ. ΚΑΝΕΛΛΟΠΟΥΛΟΥ, Φ.Ν. ΚΟΝΤΟΠΙΔΟΥ, Σ.Π. ΝΤΟΥΡΑΚΗΣ

Β΄ Πανεπιστημιακή Παθολογική Κλινική, Γενικό Νοσοκομείο Αθηνών «Ιπποκράτειο», Αθήνα

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Η κληρονομική σφαιροκυττάρωση αποτελεί την πιο συχνή κληρονομική μορφή διαταραχής της ερυθροκυτταρικής μεμβράνης και το συχνότερο αίτιο χρόνιας κληρονομικής αιμολυτικής αναιμίας. Οφείλεται σε ελλείψεις πρωτεϊνών μεμβράνης που έχουν ως αποτέλεσμα την εμφάνιση αστάθειας του κυτταροσκελετού. Παρουσιάζεται ενδιαφέρον περιστατικό που αφορά σε ένα νεαρό άνδρα, ηλικίας 32 ετών, ο οποίος εισήχθη στην κλινική λόγω άλγους στο δεξιό υποχόνδριο, ίκτερο και υπέρχρωση των ούρων. Στον ασθενή τέθηκε η διάγνωση της κληρονομικής σφαιροκυττάρωσης από έλλειψη πρωτεΐνης 3, ενώ το κλινικό σύνδρομο της εισαγωγής αποδόθηκε σε αποφρακτικό επεισόδιο του χοληδόχου πόρου. Στο παρόν άρθρο συζητούνται οι εργαστηριακές μέθοδοι που καθορίζουν τη διάγνωση και γίνεται αναφορά στους θεραπευτικούς χειρισμούς των ασθενών με κληρονομική σφαιροκυττάρωση. Η αναζήτηση της κληρονομικής σφαιροκυττάρωσης δεν πρέπει να παραλείπεται στη διερεύνηση της χρόνιας αιμόλυσης, αφού λόγω της ποικιλίας των κλινικών εκδηλώσεων της νόσου μπορεί να παραμένει ασυμπτωματική και αδιάγνωστη, ακόμη και σε μεγάλες ηλικίες.

Λέξεις ευρετηρίου: Αιμολυτική αναιμία, Πρωτεΐνη 3, Σφαιροκυττάρωση, Υπερχολερυθριναιμία, Χολολιθίαση

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Corresponding author:

T. Kanellopoulou, Second Department of Internal Medicine, "Hippokration" General Hospital of Athens, Medical School, University of Athens, 114 Vassilisis Sofias Ave., GR-115 27 Athens, Greece
e-mail: theokanel@gmail.com