

A Case of Congenital Microspherocytosis Requiring Early Splenectomy

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Microspherocytosis is known as a hallmark of hereditary spherocytosis (HS) which is one of the most common hemolytic anemias with a prevalence of one per 5000, and is inherited as Mendelian dominant. In this disorder, the patient's red cells become spheroidal, osmotically less resistant in the peripheral circulation, and are selectively trapped in the spleen, but survive normally after splenectomy. The exact mechanism for the formation of microspherocytosis has not been elucidated, although extensive investigations demonstrate that HS red cells are intrinsically defective and the patient's spleen does "condition" the metabolically abnormal red cells. The authors report here, one case of severe microspherocytosis in which changes in facial bone structure and transfusion dependency are noted, and an early splenectomy is indicated.

Key Words: Microspherocytosis, osmotic fragility test, autohemolysis test, acidified glycerol lysis test, spectrin, actin

Hereditary spherocytosis is one of the most common forms of dominantly inherited anemia. It is a single gene disorder characterized by anemia, jaundice and splenomegaly. The intrinsic defect of the red cells may be probably due to changes in the RBC membrane especially spectrin and protein 4.1. Microspherocytes are defined as small spheroidal red cells with diameters less than 75 percent of those of discocytes at the same specimen and often decrease in number after splenectomy (Sugihara *et al.* 1984).

The authors experienced one case of congenital microspherocytosis which was confirmed by the presence of microspherocytes in the peripheral blood smear, osmotic fragility test, acidified glycerol lysis test showing severe anemia but was much improved after splenectomy.

CASE REPORT

A 22 months old male baby was admitted to

Severance Hospital for splenectomy. Past history revealed that he was transferred on 13 days after birth from a local clinic to Severance Hospital due to continued severe anemia in spite of frequent transfusion. The patient was full term, vaginally delivered and weighed 2.85 kg. The baby was meconium stained but the Apgar score was 8 in one minute, 9 in 5 minutes.

During the first admission, the patient was in relatively good general condition. His skin was warm and dry with no petechiae, or purpuric skin rashes. The conjunctiva was pale and no cervical lymph nodes were palpable. Breathing sounds were clear and the heart beat was regular without a murmur. The abdomen was soft and flat, but the liver was palpable 3 cm below the the right costal margin, and the spleen was palpable 3 cm below the left costal margin with a firm consistency. Sucking and crying were good.

Peripheral blood findings revealed white blood cells 16,000/mm³, platelet count 560,000/mm³, hemoglobin level 9.2 gm/dl, which was much lower compared with 12.4 gm/dl at birth. The mean corpuscular volume (MCV) was relatively low, 79.8 fL, the mean corpuscular hemoglobin (MCH) was 27.6 pg, and the mean corpuscular hemoglobin concentration (MCHC) was 34.6 gm/dl (Table 1). The amount of microspherocytes found on the peripheral blood film was more than 40% (Fig. 1), and the reticulocyte

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Table 1. CBC, Peripheral Blood Smear and Laboratory findings

Lab.	Age	At birth	7 months	1 10/12 years *	2 2/12 years
CBC					
WBC/mm ³		16800	10300	10300	25100
Hb gm/dl		12.4	6.7	4.4	11.9
Hct %		33.5	18.7	11.9	34.1
MCV (fl)		79.8	60.6		
MCH (pg)		27.6	22.1		
MCHC (%)		34.6	36.4		
Reticulocyte (%)		20	4.2	2.0	1.1
Microspherocyte (%)		40			15
serum Bilirubin (mg%)		15.9	1.0	2.7	
serum Cholesterol (mg%)		90.5	75	100	

*splenectomy performed

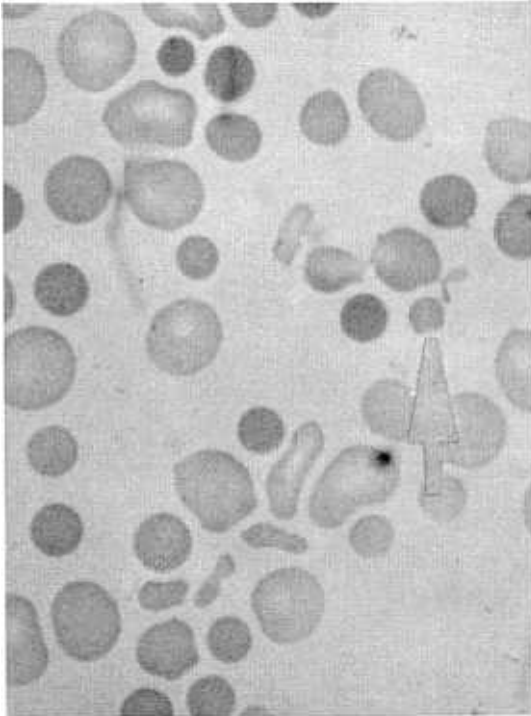


Fig. 1. The peripheral blood finding before splenectomy. About 40% of red cells are microspherocytes.

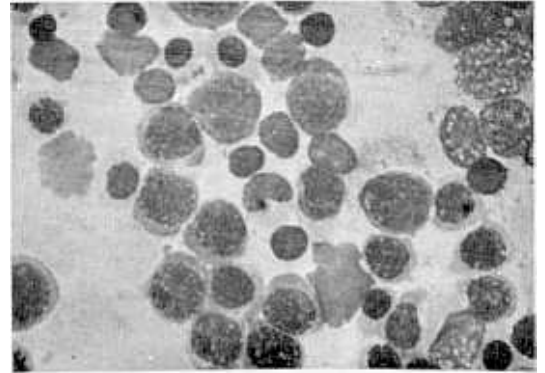


Fig. 2. The bone marrow finding shows erythroid hyperplasia.

Patient's blood type was B+, mother's blood type was AB+. Cerebrospinal fluid (CSF) finding was normal and no organism grew on both CSF and blood culture. Prothrombin time and partial thromboplastin time were within normal ranges. Serum iron was elevated, 182 µg/dl, unsaturated iron binding capacity was 143 µg/dl. TORCH study and both mother and patient's VDRL tests were done to find out perinatal infections but all of them were negative. Direct and indirect Coombs' test were negative, and the H₂O₂ hemolytic sensitivity test to rule out vitamin E deficiency was negative. PK (pyruvate kinase) and G-6-PD (glucose-6-phosphate dehydrogenase) were all within normal ranges. Hemoglobin electrophoresis to rule out hemoglobinopathies was negative.

Bone marrow examination showed no significant findings except erythroid hyperplasia (Fig. 2). Incubated osmotic fragility test and acidified glycerol lysis test were positive (Fig 3 and 4). Despite frequent

count was 20%.

Serum electrolytes and Ca, P, BUN, Cr, SGOT, SGPT were all within normal ranges. Serum bilirubin level was 15.9 mg/dl at birth but it fell to 3.5 mg/dl. Serum cholesterol level was decreased to 75 mg%.

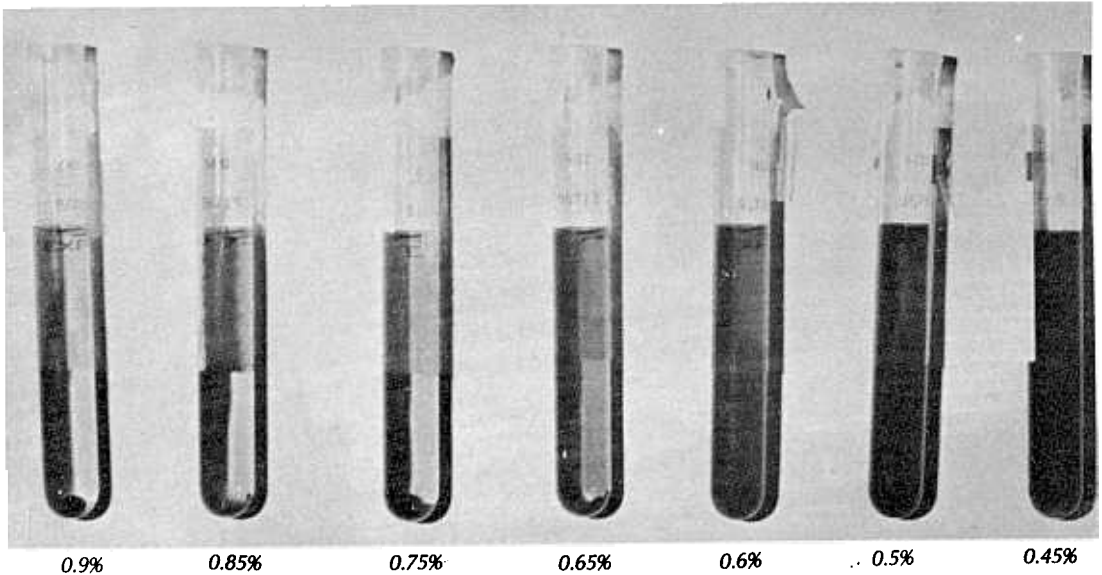


Fig. 3. The hemolysis began at 0.85% saline and was completed at 0.5% saline in the osmotic fragility test.

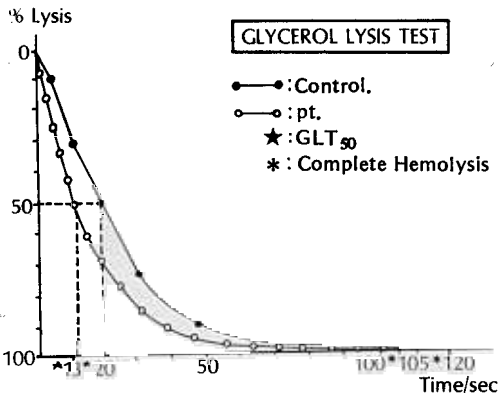


Fig. 4. The positive result of acidified glycerol lysis test.



Fig. 5. The facial findings showing frontal bossing, prominent malar eminence and exposure of upper incisor teeth.

transfusions of about once a week, the hemoglobin level was as low as 5.7 gm/dl at 1 month of age. Of the family history, the patient was first only baby and there was no known hereditary or hematologic diseases. Peripheral blood studies of both parents revealed no abnormalities.

During the second admission to perform splenectomy at 22 months of age, his body weight was 11 kg (50-75 percentile), length 82 cm (50-75 percentile), head circumference 48.5 cm (75-90 percentile), and general condition was good. On physical examination, the patient was pale and showed marked maxillary, frontal bone protrusion and exposed up-

per incisor teeth (Fig. 5). These findings were not prominent during the first admission. Breathing sounds were clear in both lung fields and a Grade II/VI functional heart murmur was heard. The liver was palpable 3 cm below the right costal margin and the spleen was palpable 8 cm below the left costal margin with

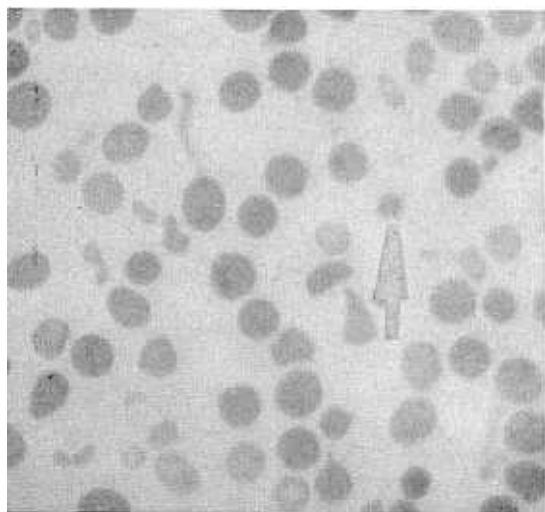


Fig. 6. Peripheral blood finding 4 months after splenectomy. Spherocytes still remained but micro-spherocytes apparently decreased in number.

platelet count gradually increased to more than 1,000,000/mm³, but the hemoglobin level could be maintained at 11-12 gm/dl and the microspherocytes decreased to about 15% (Fig.6) after the operation. Thereafter the patient received Benzathin Penicillin 600,000 unit IM injection every three weeks.

DISCUSSION

Hereditary spherocytosis (HS) was first described in 1871 by Vanlier and Masius; and Minkowaki reported osmotic fragility of HS red cell in 1900. Thereafter it was elucidated that decreases in surface area in relation to the volume may be a contributing factor in RBC destruction. In 1940, splenectomy was found to be effective in treatment. In 1960s, decrease in membrane lipids was thought to be the major cause of spherocytosis (Cooper and Jandle 1969; Reed and Swisher 1966). In 1970s, analysis of membrane protein became a major subject and it was found that

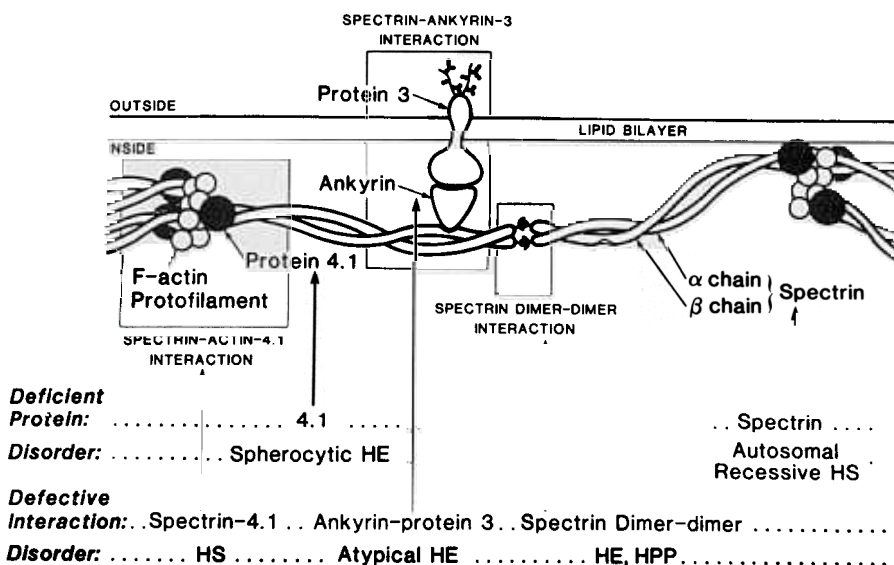


Fig. 7. Schematic illustration of the sites of skeletal defects in hereditary spherocytosis (HS), elliptocytosis (HE), and pyropoikilocytosis (HPP). (Palek J and Lux SE: Semin Hematol 20:190, 1983)

a firm consistency. Hemoglobin level was 4.4 gm/dl, reticulocyte was 2.0%, serum electrolytes were all within normal ranges and bilirubin level was 2.7 mg/dl. During surgery, no accessory spleen was found and the resected spleen weighed 210 gm with a congestion of red pulp and empty sinusoidal space. The

hereditary spherocytosis involves an abnormality in protein of the red cell membrane, especially spectrin (Jacob *et al.* 1971). At the end of 1970s and 1980s, skeletons of RBC membrane were closely analyzed and it was found that the defect in interaction of spectrin, actin, protein 4.1 (Cohen *et al.* 1980; Tyler *et al.*

1980) may play a role in morphologic changes of RBC which may lead to hemolytic anemia (Wolfe *et al.* 1982).

Hereditary spherocytosis is a single gene disorder inherited as autosomal dominant in 75-80% of cases, but 25% of cases may occur sporadically (Morton *et al.* 1982). Recessively inherited spherocytosis is a severe, though rare, disease with marked anemia, massive splenomegaly and frequent episodes of jaundice as recently described in two sisters (Agre *et al.* 1982). The parents and all ancestors examined had no evidence of red cell disorder. The osmotic fragility of these red cells was usually severe. The blood smear showed that all the red cells are microcytic most being spherocytic with acanthocyte, bizarre forms and a few nucleated red cells. Both had striking clinical improvement but spherocytosis persisted after splenectomy. The patients' red cell membrane had less than 50% of the normal amount of spectrin when measured in both ghosts and whole red cell lysates. Protein assay of these patients were not done but these patients had a severe form of spherocytosis, and it may be probably due to an inadequate amount of spectrin, and the manner of inheritance was probably Mendelian recessive. As suggested by Kimberling *et al.* in 1978 and Bass *et al.* in 1983, hereditary spherocytosis locus was located on or near the short arm of chromosome 8. Here the presented case is very similar to Agre's report because there is no known family history and the severity also required splenectomy.

The incidence of hereditary spherocytosis is one per 5000, similar to congenital hypothyroidism and there are many reported cases in Korea. Ko *et al.* (1977) reported 47 cases of HS, family history were found in 76.6%, similar to Morton's report. The age distribution showed 100 days to 53 years with the mean age of symptom onset 7.8 years. They suspected reasons why the diagnosis was delayed was primarily due to the fact that most of the patients have no visible or severe symptoms. Acute crisis was suspected in 34% of cases, hemolytic and aplastic were same in number but none had megaloblastic crisis. Kim *et al.* (1982) reported 17 cases with age distribution of 9 months to 19 years but the patients who underwent splenectomy were all above 6 years of age. But there were no reported case of microspherocytosis requiring early splenectomy in Korea.

There are two clear determinants of manifestation of hereditary spherocytosis: firstly, the defect intrinsic to the red blood cell and secondly, the exacerbating, destructive role of the spleen.

The morphology and osmotic fragility of RBC is related to the shape. There is a disproportion between surface area and volume. The volume is normal but

the surface area is less. When there is defect in the membrane, RBC shape will become spheroidal. Although the mechanism is not clear, it is well established that the red cell membrane in hereditary spherocytosis is abnormally permeable to the passive influx of sodium (Mohler 1965). If the HS erythrocyte compensates for this increased influx of sodium by increasing active efflux, then HS red cells should utilize greater amounts of ATP and glucose than normal red cells to provide the energy for increased active cation transport. When the glucose is depleted, sodium leakage will be increased which may attribute to the morphologic changes to spheroidal. But there was no correlation between sodium influx and the hemolytic tendency of hereditary spherocytes when the latter was measured by the survival time in compatible normal recipients (Wiley 1970). The osmotic fragility and shape of HS cells could also be improved if iron deficiency anemia was imposed by phlebotomy, but the cell survival remained unchanged, so the osmotic fragility is not the fundamental determinant of hereditary spherocyte survival (Crosby and Conrad 1960). The red cell membrane, like all other eukaryotic plasma membrane, is composed of a bilayer of several different classes of lipids along with a variety of protein molecules. About half of the red cell membrane mass is accounted for by lipids (primarily phospholipid and cholesterol) and glycoprotein. Somewhat more than 30% (by weight) of the proteins are deeply embedded in the lipid bilayer (integral proteins), while another 50% are associated with the membrane surface (peripheral proteins). Peripheral proteins, which include those of the membrane skeleton, can be released from the lipid bilayer under the influence of environmental manipulation or mild chemical perturbation (Cohen 1983). Nearly a decade ago, intensive efforts to map the transmembrane distribution of the major red cell membrane proteins led to the conclusion that all of the red cell membrane peripheral proteins were exposed only at the cytoplasmic membrane surface. By contrast, the major integral membrane proteins (most notably Band 3, then anion channel, and glycophorin) span the bilayer. Of the six or seven major proteins at the cytoplasmic surface, four—spectrin, actin, protein 4.1, and protein 4.9—make up the membrane skeleton (Cohen 1983).

The primary molecular lesion appears to reside in the membrane skeleton and, in some patient, is expressed as an increased susceptibility to membrane fragmentation and a loss of membrane surface. The progressive loss of membrane surface causes HS red cell to become increasingly spheroidal, osmotically fragile and rigid, and subjects it to detention in the splenic cords where the metabolically inhospitable en-

environment and the high concentration of macrophages combine, in a still uncertain manner, to accentuate the basic membrane defect and enhance spheroidicity. This process is known as splenic conditioning. Conditioned red cells appear as microspherocytes in the peripheral circulation and are particularly susceptible to recapture and destruction in the spleen and other parts of the reticuloendothelial system (Lux 1983).

Spectrin, a major skeletal protein and account for 50 to 75 percent of the skeletal mass, is important for the maintenance of phospholipid asymmetry (Haest 1982; Williamson *et al.* 1982). It contains two enormous polypeptide chains that are structurally and functionally distinct: Protein band 1 (alpha chain; 240,000 daltons) and 2 (beta chains 220,000 daltons). These chains are linear arrays of organized helical domains linked by short protease-sensitive regions. They are aligned side by side in the heterodimer, forming a long, slender, twisted wormlike molecule. The protein is highly flexible and assumes a variety of conformations, an unusual property that may be critical for normal membrane pliancy (Lux 1983) (Fig. 7)

Red cell actin is very similar to other actins, both structurally and functionally. But unlike the actin in other cells, red cell actin appears to be organized as short, double helical F-actin filaments about 10 to 20 monomers long. There is some evidence that the state of actin polymerization is functionally important to the red cell since compounds that inhibit actin polymerization increase membrane flexibility, while compounds that promote polymerization rigidify the membrane. Spectrin tetramers are bivalent and can cross-link actin filaments; binding is weak and ineffective in the absence of protein 4.1 (Cohen *et al.* 1981)

Protein 4.1, a globular protein (78,000 daltons, sphere shape) is a core skeletal component and is necessary for normal skeletal stability (Cohen and Foley 1980). It binds tightly to spectrin at the tail end of the molecule, very near the actin binding site (Mueller and Morrison 1980).

Ankyrin is a large, pyramidal-shaped protein that serves as the high affinity binding site for the attachment of spectrin to the inner membrane surface. Ankyrin is bound to the cytoplasmic portion of protein 3, the true anchor for the membrane skeleton (Bennett and Stenbuck 1980).

Protein 3 (Anion exchange protein), the major cell membrane protein, is a 93,000 dalton transmembrane glycoprotein that probably exists in the membrane as a noncovalently linked tetramer. Six to seven percent of the population is heterozygous for a slightly larger, but apparently functionally normal, variant. Like other integral proteins, protein 3 contains several structurally and functionally unique domains. The hydrophobic

intramembranous domain forms the physiologically important anion exchange channel that enables the red cell to exchange Cl^- for HCO_3^- and transport CO_2 from the tissue to the lungs. The cytoplasmic domain functions as a binding site for a larger number of red cell proteins including: hemoglobin; protein 4.2; ankyrin; and the glycolytic enzymes (Lux 1983).

Biochemical studies of HS membrane lesion in man have so far led to the identification of two distinct molecular defects: partial spectrin deficiency and defective binding of spectrin to protein 4.1. Goodman *et al.* (1972) have reported that two kindreds studied show this binding defect. In their study, spectrin and protein 4.1 are present in normal quantities in the ghosts of affected patients, but isolated spectrin binds with equal affinity only 55% to 65% as much protein 4.1 as normal spectrin. Wolfe and coworkers (1982) have also shown binding of protein 4.1 by spectrin to be abnormal in one of six kindreds with HS. They found the binding of normal 4.1 by spectrin from all four patients in the affected kindreds was reduced approximately 40%. The HS spectrin was separated into two types by affinity chromatography on immobilized protein 4.1: 40% did not bind to 4.1, while the remaining 60% bound 4.1 normally. The conclusion from these studies was that a subset of kindreds with typical autosomal HS makes two types of spectrin in approximately equal quantities: one type has normal 4.1 binding properties, while the other, the product of the autosomal dominant gene, does not bind protein 4.1, there producing membrane instability and the chronic hemolytic syndrome characteristic of HS.

In addition to the protein-protein associations discussed above, it is also likely that the cytoskeletal proteins have important associations with membrane lipids. It is possible, for example, that some of the binding of protein 4.1 to red cell vesicles maybe accounted for by association with lipids.

The membrane of HS red cells are deficient in cholesterol and phospholipid prior to splenectomy. The relative proportions of cholesterol and the various phospholipids are normal in HS red cells, and the phospholipid show the usual transmembrane asymmetry. It has been reported that very long chain fatty acids are missing from certain classes of phospholipids, but this has not been confirmed (Aloni 1975). Controversy also exists over whether membrane lipid fluidity is or is not normal. At the present time it is unclear whether these differences are due to technical factors or to genetic heterogeneity of the disease. Even if real, it seems likely that in the affected patients the changes in fatty acid composition and membrane fluidity are secondary to an underlying membrane protein defect. After splenectomy, lipid content is similar

to that of normals with spleen, but still deficient when compared with splenectomized controls. There is a similar lipid deficit in the cells of autoimmune hemolytic anemia and out-dated blood, so this does not appear to be unique for HS (Becker and Lux 1985). Some suggested that depressed phosphorylation for the small polypeptide may play a role in the pathogenesis of HS (Wolfe *et al.* 1982; Thompson *et al.* 1981). However, kinases isolated from the spherocytes exhibit normal phosphorylation of exogenous substrates. Thus, phosphorylation apparatus is probably intact in HS, but the function of the phosphorylated proteins may be defective (Becker and Lux 1985).

The spleen is said to "condition" hereditary spherocytes, causing increased spheroidicity and osmotic fragility. Conditioning also cause lipid loss by an unknown mechanism. The spleen may adversely affect hereditary spherocytes by physical entrapment, an environment detrimental to red cell metabolism or membrane structure, and phagocytic damage.

Normal erythrocyte become spheroidal when out of survival time and are easily destroyed in the spleen, so it is assumed that life span of erythrocyte of HS is much shortened to about 40 days (Wiley 1970). Normal erythrocyte encompass the deformability which enable to pass the splenic sinus (3-5 μm) easily. Spherocytes may be trapped in the spleen because they are unable to deform sufficiently to pass through the spaces between the endothelial cells that form the walls of the sinuses. The reduced deformability leads to entrapment of spherocytes. The entrapped cells also encounter the poor condition such as low PH, less glucose, low O_2 level, low ATP level and are easily destroyed (Lux 1983). Under these splenic condition the membrane of spherocyte would be severely damaged and contribute to the formation of microspherocytes. Microspherocytes were defined by Takashi *et al.* (1984) as small spheroidal red cells with diameters less than 75 percent of those of discocytes at the same specimen. They found microspherocytes disappeared concomitant to substantial normalization of plasma and red cell membrane lipid after splenectomy which may be at least one of the causative factors in pathogenesis of the formation of microspherocytosis. Microspherocytes disappeared substantially from the peripheral circulation of the HS patient after splenectomy. In contrast, cell fraction other than that of microspherocytes, such as from discocyte to spherocyte, remained almost unchanged despite splenectomy. In this presented case, plasma cholesterol is decreased to 75-100 mg% before splenectomy.

Three broad classes of severity of diseases can be

distinguished in patients with HS, mild, moderate and severe. Approximately a quarter of all patients have a mild form of the disease (Lux 1983), same as Ko reported in 1977. Hemolysis and splenomegaly are mild or absent in these patients due to successful compensation of erythroid production in bone marrow, but hemolytic destruction can become severe during illness that cause splenomegaly such as infectious mononucleosis, or at times of other physiological stress, such as vigorous exercise and pregnancy. Two-thirds of patients have moderate form with uncompensated hemolytic destruction and intermittent periods of jaundice, often associated with viral infections (Lux 1983). Splenomegaly is usually present and the spleen is variable in size. About 10% of patients are severe form, sometimes transfusion-dependent. These patients may have aplastic crises, growth retardation and the changes in facial bone structure, as this presented case.

Jaundice can be caused by hereditary spherocytosis in the neonatal period (Stamey and Diamond 1957), prominent in 48 hours of birth and may require phototherapy in most of the patients. Rarely, exchange transfusion may also be required to prevent kernicterus. This presented case showed total bilirubin level no more than 15.9mg/dl, did not require exchange transfusion.

Two major types of crises occur in HS; hemolytic and aplastic. A hemolytic crises can be precipitated by infection, stress or folic acid deficiency and is characterized by increased unconjugated bilirubin, splenomegaly and anemia. Hemolytic crises are usually not severe and rarely require transfusion (Lux 1983). Aplastic crises are much more serious resulting in severe anemia, and may be fatal (Lux 1983). Granulocytopenia and thrombocytopenia may accompany the erythroid hyperplasia. The crises typically lasts ten to fourteen days and ends with the reappearance of reticulocytes in the peripheral blood (Owren 1948). Megaloblastic crises result when the dietary intake of folic acid is insufficient for the increased needs of erythroid HS bone marrow. They are observed during pregnancy when the need for folic acid is particularly high (Delamore *et al.* 1961).

Red cells from some patients with HS had an increased mean corpuscular hemoglobin concentration (MCHC) compared with normal (36% versus 32%) in about half of the patients owing to mild cellular dehydration. The mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) fall within the normal range, but because young red cells normally have a high cell volume, the MCV in HS is actually relatively low (Lux 1983).

Specific laboratory tests include osmotic fragility

test, 24 hr incubated osmotic fragility test, autohemolysis test and acidified glycerol lysis test. Osmotic fragility is less sensitive whereas acidified glycerol lysis test is more sensitive and specific (Gottfried et al. 1985; Zanella et al. 1980), gives clear cut results, and the required amount of specimen is only 20 ml (Zanella et al. 1980). Results are expressed as the time required for the optical density to fall to half of the initial value. This test become occasionally false positive, so Imagawa et al. (1985) devised new analyzing method i.e., decrement of a variable optical density for one minute after an adding glycerol reagent. They suggested this method showed a significant correlation with the osmotic fragility test and would be able to be used one of the screening tests for HS. Gel electrophoresis, radioimmunoassay can also be used for the analysis of membrane.

Splenectomy permits lengthening of the hereditary spherocytes and abbreviated lifespan of nearly normal generally eliminates the signs of the disease, although the intrinsic red cell abnormalities persist. It is usually performed at the age of 5–6, but in this severe case requiring frequent transfusion and changes in facial bones are noted, it is performed at the age of 22 months.

The most common complication of HS is gallbladder disease, pigment gallstones have been detected in patients as young as 3 years but are most prevalent in adolescent and adults (Rutkow 1981). The available data indicate that 55–85 percent of untreated HS patients will eventually acquire stones and that roughly half of these individuals will have symptoms of cholecystitis, or less commonly, biliary obstruction. Rutkow (1981) reported that in 58 HS patients cholelithiasis was present in only 21%, when analyzed for patients 10 years of age or older the incidence was 41%, and accessory splenic tissue was located in 17% of patients. Because lifespan of RBC is still shortened after splenectomy in HS, gallstone is found in 13% in postsplenectomized patients (Schwartz et al. 1970). Other supportive care include transfusion, folic acid administration, and pneumococcal vaccination, antibiotic prophylaxis after splenectomy.

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