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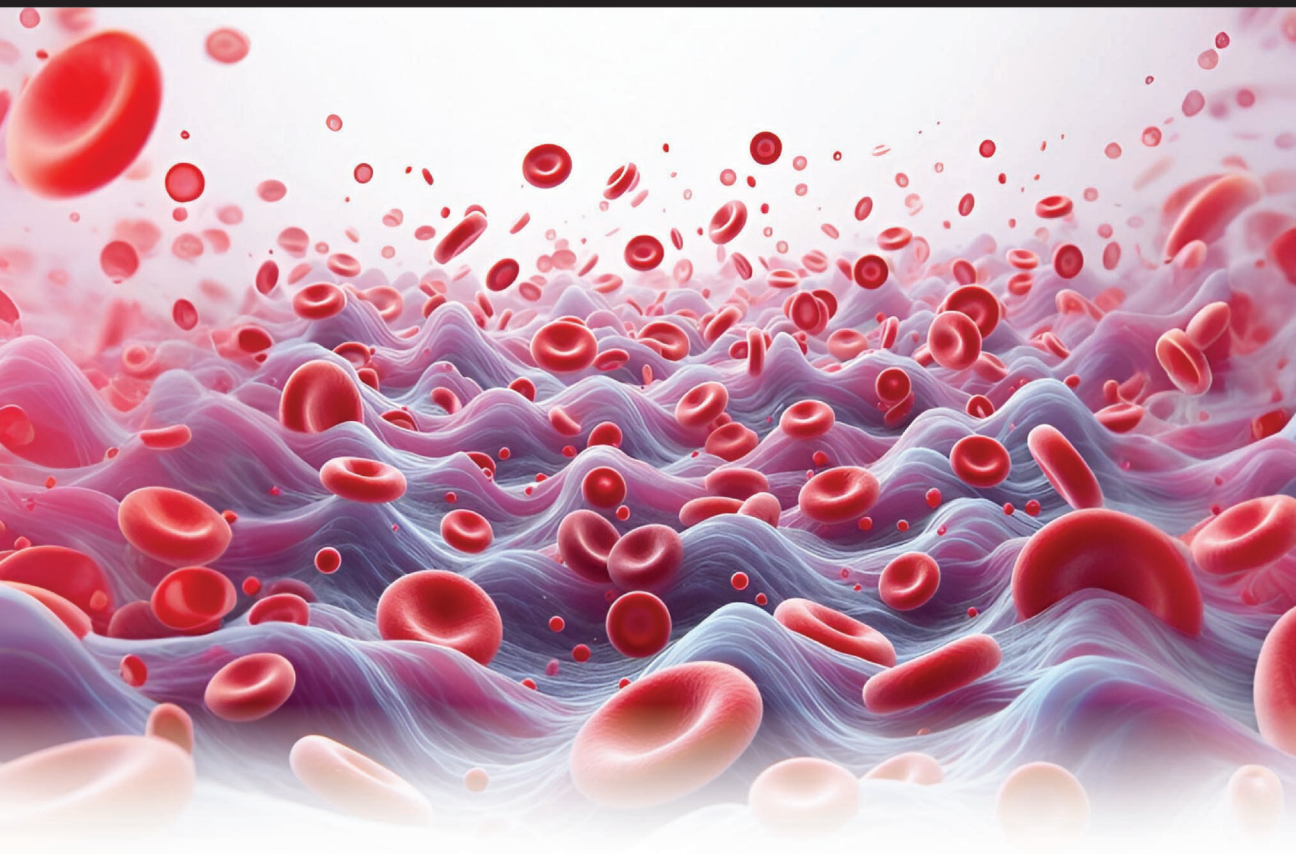

Essentials of HEMATOLOGY

Shirish M Kawthalkar

4th Edition

JAYPEE

Contents

CHAPTER

4 Anemias Due to Excessive Red Cell Destruction

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DISORDERS OF RED CELL MEMBRANE

Abnormalities in the red cell membrane causing hemolytic anemia are shown in **Table 4.1**.

HEREDITARY SPHEROCYTOSIS

Hereditary spherocytosis (HS) is a congenital hemolytic disorder characterized by an inherited defect in the red cell membrane cytoskeleton leading to the formation of spherocytic red cells. Spherocytes, being less deformable than normal red cells, are trapped and destroyed in the spleen. Spherocytes have reduced surface area to volume ratio and are osmotically fragile.

TABLE 4.1: Disorders of red cell membrane associated with hemolytic anemia.

Disorder	*Inheritance*	*Defective gene*	*Pathophysiology*	*Red cell morphology*
Hereditary spherocytosis	AD (75%), AR (25%)	Ankyrin, spectrin, band 3, protein 4.2	Defective vertical interaction between cytoskeletal proteins and lipid bilayer	Spherocytes
Hereditary elliptocytosis	AD	Spectrin, protein 4.1	Defective horizontal protein interactions	Elliptocytes (>50%)
Hereditary pyropoikilocytosis	AR	Partial spectrin deficiency	Defective horizontal protein interactions	Poikilocytes (Fragments)
Overhydrated hereditary stomatocytosis (hereditary hydrocytosis)	AD	Not known	Abnormal permeability to sodium leading to cell swelling	Stomatocytes
Dehydrated hereditary stomatocytosis (hereditary xerocytosis)	AD	Not known	Loss of intracellular potassium due to increased permeability	Target cells, red cells with hemoglobin puddled at periphery
Southeast Asian ovalocytosis*	AD	Band 3	Red cell membrane rigidity; resistance to *P. falciparum* invasion	Rounded elliptocytes with transverse ridges or longitudinal slit
Acanthocytosis (abetalipoproteinemia)**	AR	*MTTP* gene	Increase in sphingomyelin in membrane	Acanthocytic red cells

* Not associated with hemolytic anemia
** Acanthocytosis is also seen in McLeod phenotype and neuroacanthocytosis syndromes.
(AD: Autosomal dominant; AR: Autosomal recessive)

Although the most common mode of inheritance is autosomal dominant (AD), autosomal recessive (AR) transmission occurs in some cases. HS occurs in all races but has a high prevalence in people of northern European descent (1:5000) and in Japanese individuals; the prevalence may be an underestimation since milder forms of the disease are asymptomatic. Prevalence of HS in other ethnic groups is unknown.

ETIOPATHOGENESIS

Two major factors involved in pathogenesis of HS are an intrinsic defect in red cell membrane and destruction of defective red cells in spleen.

The Basic Lesion

Normally, lipid bilayer of the red cell membrane is anchored to the underlying skeleton by two major linkages. The first linkage involves interaction of ankyrin with spectrin in skeleton and band 3 in the bilayer. The second attachment between skeleton and bilayer is provided by glycophorin C and protein 4.1 (*see* **Fig. 1.15**, Chapter 1). Deficiency in any of these interactions causes weakening of contact between lipid bilayer and skeleton. As a result, areas of the lipid bilayer, which are not directly supported by the underlying skeleton, are lost from the cells in the form of small lipid vesicles. This causes a decrease in surface area of red cell relative to volume with resultant spherocyte formation.

HS may result from deficiency of the following skeletal proteins—spectrin, ankyrin, band 3, and protein 4.2 **(Table 4.2)**.

Red Cell Destruction by Spleen

Spherocytes are more rigid and less deformable than normal red cells. This nondeformability prevents the passage of spherocytes from the splenic cords into the splenic sinuses through the slit-like narrow openings. Red cells are trapped in fenestrations in the wall of splenic sinuses where blood from splenic cords enters red pulp. The red cells are retained in the red pulp for an unduly long time. In the red pulp, red cells encounter unsuitable environment in the form of low glucose and reduced pH. Metabolic deprivation of red cells accentuates membrane loss with release of small vesicles and increase in spherical shape of red cells.

TABLE 4.2: Membrane protein defects in hereditary spherocytosis.

Defective membrane protein (Defective gene)	*Prevalence in Europe and USA*	*Inheritance*	*Clinical severity*	*Morphology of red cells*	*Comments*
Ankyrin-1 (*ANK1*)	40–65%	AD, AR	Mild to moderate	Spherocytes	Most common cause of dominant HS
Band 3 (*SLC4A1*)	29–35%	AD	Mild to moderate	Pincer-like spherocytes	Also seen in SAO
β spectrin (*SPTB*)	15–30%	AD	Mild to moderate	Acanthocytes, spherocytes	Also seen in HE and HPP
α spectrin (*SPTA1*)	<5%	AR	Severe	Spherocytes, poikilocytes	Also seen in HE and HPP
Protein 4.2 (*EPB42*)	<5%	AR	Mild to moderate	Spherocytes	Common in Japan

(AR: Autosomal recessive, AD: Autosomal dominant, SAO: Southeast Asian ovalocytosis, HE: Hereditary elliptocytosis, HPP: Hereditary pyropoikilocytosis)

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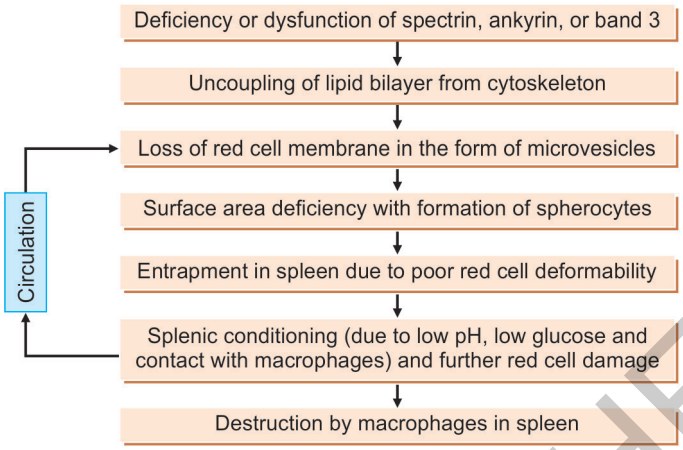

Fig. 4.1: Pathogenesis of hemolysis in hereditary spherocytosis.

Cellular dehydration occurs due to loss of potassium from the red cells. Some of the red cells may escape into the circulation where they appear as dense microspherocytes on blood films. This small population of microspherocytes is osmotically the most fragile. Most of the spherocytes are, however, retained in the red pulp where they are destroyed by macrophages. Red cells that escape are retrapped by spleen and phagocytosed **(Fig. 4.1)**.

INHERITANCE

Most of the cases exhibit autosomal dominant pattern of inheritance, while recessive transmission is less common. Usually, dominant inheritance is associated with mild to moderate disease while recessively inherited spherocytosis is associated with severe hemolysis.

CLINICAL FEATURES

Heterogeneity

The clinical presentation is markedly heterogeneous. Majority of patients present in childhood with mild to moderate anemia, intermittent jaundice, and enlarged spleen. Some other members of the family such as a sibling or a parent is similarly affected.

In some patients, clinical features are mild or entirely absent. HS may first be detected while investigating a patient who has gallstones or splenomegaly and minimal or no anemia (well-compensated hemolysis). In still other persons, the only abnormality is increased red cell osmotic fragility (OF); such persons come to attention when family members of the affected patient are investigated.

Rarely hereditary spherocytosis may present as a severe hemolytic anemia requiring regular transfusion support.

Exacerbation of Anemia

Sometimes the steady course of hereditary spherocytosis is punctuated by episodes of exacerbation of anemia or crises.

In **aplastic crisis**, there is temporary aplasia of erythroid precursors in the bone marrow resulting in sudden worsening of anemia. Infection by human parvovirus has recently been recognized as the cause. Parvovirus B19 selectively infects erythroblasts and transiently

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suppresses erythropoiesis. Infection by this virus may be asymptomatic or may manifest with fever, chills, body ache, and facial rash. Transient suppression of erythropoiesis in persons with normal red cell life span does not manifest as anemia. However, in patients with chronic hemolytic anemia in whom the red cell life span is markedly shortened, infection is associated with sudden aggravation of anemia, reticulocytopenia, and erythroblastopenia. Treatment consists of transfusion support during the aplastic phase. Increased turnover of erythroid cells due to hemolysis may lead to **megaloblastic crisis** if dietary intake of folate is insufficient. Exacerbation of hemolysis **(hemolytic crisis)** may occur during intercurrent infection due to hyperplasia of monocyte-macrophage system.

Gallstones

Pigment gallstones are common in adolescents or young adults. Sometimes HS is diagnosed incidentally during investigation of a young patient with gallstones.

Chronic Leg Ulcers

These are present in an occasional patient.

LABORATORY FEATURES

Examination of Peripheral Blood

In most patients, anemia is usually mild to moderate. MCHC is increased (>35 g/dL) due to cell dehydration, MCH is normal, while MCV is slightly low. Reticulocytosis is present. MCV is on the lower side of normal (despite reticulocytosis). Combination of MCHC (>35.4 g/dL) along with RDW (<14%) and increased percentage of hyperdense or hyperchromic cells (reported by some hematology analyzers) have been reported to be indicative of HS.

On blood smear examination, a characteristic feature is spherocytosis **(Fig. 4.2)**. A spherocyte is a red cell that is smaller in size, does not have central pallor and appears densely hemoglobinized (hyperchromic).

Some mutations are associated with typical morphological abnormalities of red cells like (1) both spherocytes and acanthocytes (mutation of β-spectrin gene), pincer- or mushroom-shaped cells (specific and sensitive for band 3 deficiency), and sphero-ovalocytes and stomatocytes (protein 4.2 deficiency in Japanese).

Spherocytes, however, are not unique to HS and are also prominently seen in other disorders such as immune hemolytic anemias (warm antibody type), ABO hemolytic disease of the newborn, hemolytic transfusion reactions, and burns.

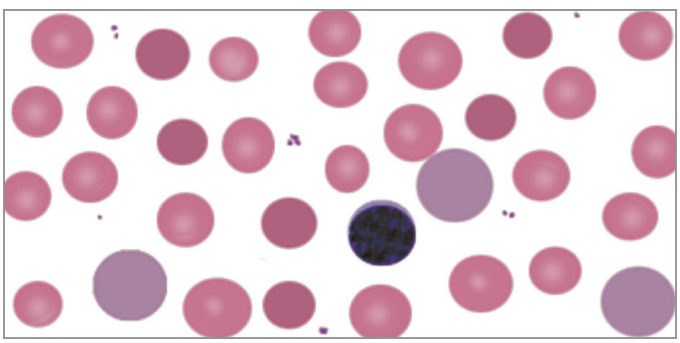

Fig. 4.2: Blood smear in hereditary spherocytosis. Spherocytes are small (compare with small lymphocyte), dense cells with no central pallor. Polychromatic cells are also increased due to hemolysis.

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In typical HS patients, markers of increased red cell destruction are present like increased reticulocytes, increased lactate dehydrogenase, increased serum indirect bilirubin, and decreased serum haptoglobin. These abnormalities, however, may be absent in mild forms of disease.

Bone Marrow Examination

This reveals erythroid hyperplasia. Aplastic and megaloblastic crises can be identified by bone marrow examination.

Osmotic Fragility Test

The osmotic fragility (OF) test is the commonly employed screening test for HS. The incubated variant of OF test (see below) is more sensitive than the unincubated test. This test determines susceptibility of red cells to hemolysis when they are subjected to osmotic stress. The red cells are suspended in decreasing concentrations of hypotonic saline solutions and the amount of hemolysis is measured. Water enters the red cells when they are placed in hypotonic saline solution. Due to their biconcave shape, normal red cells can withstand hypotonicity by increasing their volume; beyond a certain limit increase in swelling is not possible, and cells burst by discharging their hemoglobin into the supernatant. Spherocytes have a decreased surface to volume ratio and therefore, they are able to withstand less swelling than normal and are osmotically fragile, i.e., hemolysis occurs in a more concentrated solution than normal cells **(Fig. 4.3 and Box 4.1)**. There are various methods of recording results of OF test:

i. Highest concentration of saline at which hemolysis starts (normal 0.50 g/dL NaCl) and the highest concentration of saline at which it is complete (normal 0.30 g/dL NaCl).
ii. Concentration of saline showing 50% lysis (median corpuscular fragility); normal value is 0.40–0.45 g/dL NaCl. Higher value denotes increased fragility.
iii. A graph may be plotted with amount of hemolysis on vertical axis and saline concentrations on horizontal axis. Normal OF curve is sigmoid-shaped. Shift of the curve to the left indicates increased OF **(Fig. 4.4)**. A tail of the curve represents erythrocytes conditioned by spleen, which disappears following splenectomy.

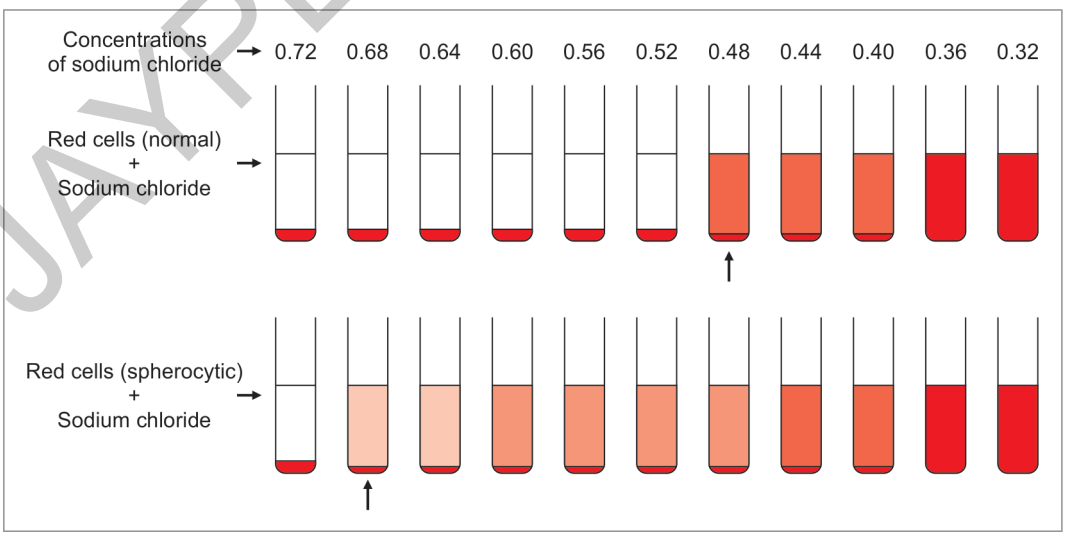

Fig. 4.3: Osmotic fragility test. In hereditary spherocytosis (lower set of tubes), hemolysis starts (up arrow) at a higher saline concentration as compared to normal (upper set of tubes).

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Box 4.1

Osmotic fragility test

- Measures ability of red cells to take up water when kept in hypotonic saline solutions
- Due to their biconcave shape, normal red cells take up more water and increase their volume by up to 70% before lysing. Owing to ↑ volume to surface area ratio, spherocytes can take up relatively little water and can withstand very little increase in their volume
- Normally red cells show beginning of hemolysis at 0.5% saline, while spherocytic red cells lyse at higher saline concentration
- OF is more marked if red cells are incubated at 37°C for 24 hours before the test
- Test is not diagnostic of HS. Positive result (increased fragility) should be interpreted in the light of clinical and other laboratory features

Limitations of Osmotic Fragility Test

- Increased osmotic fragility (OF) is due to spherocytosis, which may result from a variety of causes (HS, immune hemolysis, burns, etc.). Therefore, OF test is not specific for diagnosis of HS.
- OF test is normal in patients with mild HS having very few spherocytes in their blood. The sensitivity of the test, however, can be increased by performing the test after incubation of cells at 37°C (see below).
- Test is time-consuming, tedious; and if spherocytes are present on blood smear, adds little to diagnosis.
- Decreased osmotic fragility (increased resistance to lysis in hypotonic solutions) is seen in iron deficiency anemia, thalassemia, sickle-cell disease, and liver disease. Therefore, the test is falsely negative in the presence of the above disorders.
- OF test cannot distinguish between causes of spherocytosis.

Osmotic Fragility Test after Incubation

Sterile blood is incubated at 37°C for 24 hours followed by determination of OF in hypotonic saline solutions. During incubation, metabolic deprivation of spherocytes occurs (mainly due to decreased concentration of glucose) with resultant membrane destabilization, loss

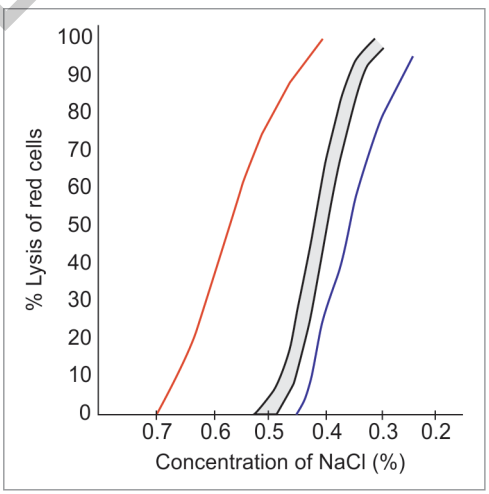

Fig. 4.4: Osmotic fragility curve. Shaded area: Normal; Red line: Hereditary spherocytosis; Blue line: Thalassemia.

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of membrane, and enhancement of spherical shape. Failure of membrane pump with accumulation of sodium and water also plays a role. The sensitivity of the test is thus increased. Incubated OF test may, however, be normal in a small number of patients with HS.

It should be noted that OF is increased in any disorder associated with spherocytosis and is thus not specific for HS. Normal OF test result does not rule out HS as it may be normal in mildly affected patients.

Autohemolysis Test

In this test, blood is incubated at 37°C for 48 hours and the degree of spontaneous hemolysis is noted. The test is performed with and without the addition of glucose. Amount of hemolysis is estimated in a colorimeter and the result is expressed as a percent lysis (normal: <4%; with glucose: <0.5%).

Red cells in hereditary spherocytosis are abnormally permeable to sodium probably because of defect in membrane skeleton. Therefore, there is compensatory increase in sodium extrusion by the membrane pump, which thus requires more ATP than normal. During in vitro incubation this causes rapid exhaustion of available red cell ATP and glucose by way of increased glycolysis. Depletion of ATP leads to failure of membrane pump and swelling of red cells as they gain sodium and water. During incubation membrane loss from the red cells is accentuated which increases the volume to surface ratio. Autohemolysis thus results with smaller red cell volume. Thus, during the 48 hours incubation period in this test, glucose exhaustion occurs due to increased utilization and autohemolysis is increased. With the addition of glucose, autohemolysis is corrected to normal levels.

Autohemolysis is also increased in deficiency of a glycolytic enzyme such as pyruvate kinase due to a block in the utilization of glucose. In this case, however, addition of glucose fails to correct the abnormality.

Acidified Glycerol Lysis Time

This test assesses the rate of lysis of red cells and the result is expressed as the length of time needed for 50% lysis ($AGLT_{50}$). Normal blood requires more than 30 minutes for 50% lysis. In HS, the time required for lysis is considerably shortened (25–150 seconds) since spherocytes (due to their high volume to surface area ratio) tolerate swelling for a lesser duration than normal cells. Along with hypotonic saline solution, in the reagent system, glycerol is used which slows the rate of movement of water across the red cell membrane; this allows easier measurement of time required for lysis.

Compared to OF test, this test is simple and rapid. But it is not diagnostic of hereditary spherocytosis since it is also positive in autoimmune hemolytic anemia, chronic renal insufficiency, leukemias, and during gestation.

Hypertonic Cryohemolysis Test

This recently introduced test is reported to be more specific for diagnosis of HS than OF test. In this test, percent cryohemolysis is observed after transferring cells from 37 to 0°C for 10 minutes. In HS, percent cryohemolysis is more (>20%) as compared to that in normal (3–15%).

Eosin-5-Maleimide (EMA) Binding Test

This is a rapid flow cytometric screening test. Fluorescently labeled eosin-5-maleimide (EMA) binds to band 3 and Rh-related proteins with a 1:1 stoichiometry on red cell membrane. The

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relative amount of fluorescence correlates with the amount of EMA binding and is analyzed by flow cytometry. Reduced fluorescence intensity of EMA-labeled red cells is observed in HS due to band 3 deficiency, and also in HS associated with spectrin and ankyrin deficiency. The test has high sensitivity and specificity for identification of HS. However, the result is normal in mild HS.

Osmotic Gradient Ektacytometry

This test measures deformability (extent of shape change) of red cells under a continuous osmotic gradient and a known shear stress and generates a deformability index (DI) profile. DI profile provides information about cell water content and heterogeneity of deformability. Distinct deformability profiles are obtained in red cell membrane disorders. This test needs highly sophisticated equipment that limits its availability. This test is considered as the reference method for differential diagnosis of red cell membrane disorders. The parameters evaluated are:

- **Di_{max} (Maximal deformability index):** It represents maximum cellular deformability of the red cell population.
- **O_{min}:** Hypotonic osmolality at which 50% of red cells are hemolyzed and represents surface area to volume ratio.
- **O_{hyper}:** Reflects hydration status of red cells; it is the hypertonic descending part of the curve.

Laboratory tests for red cell membrane disorders are shown in **Table 4.3**.

Identification of Deficient Cytoskeletal Protein

It may be possible to identify the deficiency of a specific red cell membrane protein. The usual method consists of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of red cell membrane proteins followed by quantitation of separated proteins by densitometric tracing. The results are expressed as ratios of cell membrane proteins to band 3. Deficiencies

TABLE 4.3: Characteristic features of hereditary red cell membrane disorders.

Parameter	*HS*	*HE*	*HPP*	*SEAO*	*DHS*	*OHS*
Inheritance	AD, AR	AD	AR	AD	AD	AD
Red cell indices	↑MCHC	↑MCH (severe cases)	↓↓MCV	MCV-Normal	↑MCHC	↑MCV, MCHC↓
Blood smear	Spherocytes	Elliptocytes	Microspherocytes, micropoikilocytes	Ovalocytes	Stomatocytes <20%	Stomatocytes >20%
EMA binding test	↓	Normal	↓↓	↓	Normal or ↑	Normal or ↑
Ektacytometry	• Di_{max} ↓ • O_{min}-R • Ohyper-L	• Di_{max} ↓ • O_{min}-Normal • Ohyper-Normal	• Di_{max} ↓↓ • O_{min};L • Ohyper-L	Di_{max} close to null	Osmotic deformability profile shifted to left	Osmotic deformability shifted to right
Molecular defect	ANK1, SPTB, SPTA1, SLC4A1 (band 3), EPB42 (protein 4.2)	SPTA1, SPTB, EPB41 (protein 4.1R)	SPTA1	SLC4A1 (band 3)	PIEZO1, KCNN4	RHAG

(HS: Hereditary spherocytosis; HE: Hereditary elliptocytosis; HPP: Hereditary pyropoikilocytosis; SEAO: Southeast Asian ovalocytosis; DHS: Dehydrated hereditary stomatocytosis; OHS: Overhydrated hereditary stomatocytosis; AD: Autosomal dominant; AR: Autosomal recessive; MCHC: Mean cell hemoglobin concentration; MCH: Mean cell hemoglobin; MCV: Mean cell volume; EMA: Eosin-5-maleimide).

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of spectrin, ankyrin, band 3, protein 4.2, and some other membrane proteins can be identified by this method in the majority of patients.

A more sensitive method of membrane protein quantitation is radioimmunoassay.

Deficient proteins can also be identified by DNA analysis.

DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

Diagnostic features of HS are shown in **Box 4.2**.

DIFFERENTIAL DIAGNOSIS

Diagnosis of hereditary spherocytosis is usually easily made on the basis of mild to moderate anemia with spherocytosis, splenomegaly, jaundice, increased OF, and evidence of hereditary spherocytosis in the first-degree relative.

Sometimes anemia is mild or absent and the patient may first present with isolated splenomegaly, gallstones, or "aplastic crisis." Clinical evaluation and examination of blood film for spherocytes are necessary for correct diagnosis.

HS may have to be differentiated from other causes of spherocytosis including autoimmune hemolytic anemia and ABO hemolytic disease of the newborn. Differentiation is made by antiglobulin (Coombs') test and family studies.

TREATMENT

To plan appropriate treatment, it is necessary to grade HS into mild, moderate, and severe forms (based on hemoglobin, reticulocyte count, and serum bilirubin). Treatment of severe HS is splenectomy. Splenectomy corrects hemolytic anemia (though underlying skeletal defect and spherocytosis persist) and prevents complications such as gallstones and risk of aplastic crisis. Although splenectomy is associated with increased life-long risk of sepsis from pneumococci and other encapsulated bacteria, risk is more in children and for this reason splenectomy is deferred until the child is 6 years old and is carried out only if the disease is severe or moderate. The risk of postsplenectomy infections can be reduced by immunizing children with polyvalent pneumococcal, *H. influenzae,* and meningococcal vaccines and by penicillin prophylaxis. Other complications of splenectomy include increased risk of thrombosis and vascular complications like pulmonary hypertension and cardiovascular disease.

Box 4.2

Diagnosis of hereditary spherocytosis

- Presentation in childhood with anemia, jaundice, and splenomegaly
- Positive family history (autosomal dominant) of jaundice, gallstones, or splenectomy
- Spherocytosis on blood smear
- Reticulocytosis
- Red cell indices: ↑MCHC, ↓MCV
- Negative direct antiglobulin test
- Screening test: OF test, AGLT, cryohemolysis, EMA binding test, osmotic gradient ektacytometry
- Confirmatory test: Electrophoretic analysis (SDS-PAGE) of red cell membrane proteins or DNA analysis (Diagnosis does not require screening test or confirmatory test if other typical features listed above them are present)

Note: As presentation of HS is relatively asymptomatic, this diagnosis should be excluded in patients with isolated splenomegaly, unexplained unconjugated hyperbilirubinemia, gallstones in children and young adults, severe anemia during pregnancy (due to crises), or transient fall in hemoglobin during acute infections (due to hemolytic crisis).

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Administration of folate is necessary in moderate or severe diseases to prevent megaloblastic anemia due to increased erythrocyte turnover.

HEREDITARY DISORDERS OF HEMOGLOBIN

GENERAL FEATURES AND APPROACH TO DIAGNOSIS

Inherited disorders of hemoglobin are the most common genetic disorders in the world. More than 300,000 infants are born every year worldwide with these disorders and many of the affected children die before the age of 5 years. Depending on the nature of genetic defects, they can cause chronic hemolytic anemia or may remain asymptomatic. These disorders are more common in Mediterranean region, Africa, and Southeast Asia. In certain countries, they constitute a public health problem.

CLASSIFICATION

Inherited disorders of hemoglobin due to structural alteration of the globin polypeptide chain (qualitative defect) are called as hemoglobinopathies. Majority of hemoglobinopathies result from substitution of a single amino acid in globin chain due to a point mutation in the β-globin gene. The most frequent hemoglobinopathies are HbS, HbC, and HbE. Inherited disorders of hemoglobin due to reduced synthesis of one or more globin chains (quantitative defect) are known as thalassemias. (However, some forms of thalassemias are characterized by reduced synthesis of structurally defective globin chains and are called thalassemic hemoglobinopathies; these include HbE, Hb Lepore, and Hb Constant Spring). The two common types of thalassemias are α and β.

Both hemoglobinopathies and thalassemias are common in India **(Box 4.3 and Table 4.4)**.

Classification of hemoglobinopathies and thalassemias is as follows:

I. **Hemoglobinopathies:** Mutations alter the amino acid sequence causing qualitative defect in globin chain
   a. Abnormal hemoglobin polymerization: HbS
   b. Abnormal hemoglobin crystallization: HbC
   c. Low oxygen affinity: Hb Kansas
   d. High oxygen affinity: Hb Zurich
   e. Unstable hemoglobins
   f. M hemoglobin

II. **Thalassemias:** Mutations cause quantitative deficiency of globin chain
   a. α-thalassemias: α^0, α^+
   b. β-thalassemias: β^0, β^+
   c. δβ-thalassemias
   d. γδβ-thalassemias
   e. εγδβ-thalassemias

Box 4.3

Hereditary disorders of hemoglobin in India.

- **β-thalassemias:** North, West, and East India, with highest prevalence in Sindhis, Punjabis, Gujaratis, and Bengalis
- **HbS:** Central and Southern India
- **HbD:** North India (especially Punjab)
- **HbE:** Northeast India

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TABLE 4.4: Hereditary disorders of hemoglobin prevalent in India.

Disorder	*State*	*Clinical phenotype*
A. Hemoglobinopathies		
HbS	Heterozygous Homozygous	Asymptomatic Severe
HbD^{Punjab}	Heterozygous Homozygous	Asymptomatic Mild
HbE	Heterozygous Homozygous	Asymptomatic Asymptomatic
B. Thalassemias		
β-thalassemia minor	Heterozygous	Asymptomatic
β-thalassemia major	Homozygous	Severe
C. Double heterozygous states		
HbS/β+ thalassemia		Mild
HbS/β° thalassemia		Like sickle-cell anemia
HbS/HbD^{Punjab}		Like sickle-cell anemia
HbE/β-thalassemia		Like β-thalassemia major
HbD^{Punjab}/β-thalassemia		Mild

f. Thalassemic structural variants (Thalassemic hemoglobinopathies): Hb Lepore, HbE, Hb Constant Spring
g. Hereditary persistence of fetal hemoglobin (HPFH)
h. Dominant thalassemia
i. β-thalassemias associated with structural hemoglobin variants: HbS/β-thalassemia, HbC-β-thalassemia, HbE-β-thalassemia

Clinically, thalassemias are classified as follows:

I. **Classification of α- and β-thalassemias according to clinical severity:**
   1. α-thalassemias:
      a. α-thalassemia silent carrier (single α-gene deletion)
      b. α-thalassemia trait/minor (double α-gene deletions)
      c. HbH disease (triple α-gene deletions)
      d. Hb Bart's hydrops fetalis syndrome (absence of α-genes)
   2. β-thalassemias:
      a. β-thalassemia minor: Genotype β^0/β or β^+/β
      b. β-thalassemia intermedia: Genotype β^+/β^+, δβ/δβ, $\beta^0/\delta\beta$, dominant β-thalassemia, hemoglobin H disease
      c. β-thalassemia major: Genotype β^0/β^0, β^+/β^+ (some cases),

II. **Classification of thalassemias based on transfusion requirements:**
   1. Nontransfusion-dependent thalassemias or NTDT (occasional or intermittent transfusions required)
      a. β-thalassemia intermedia
      b. Mild or moderate HbE/β-thalassemia
      c. HbH disease
   2. Transfusion-dependent thalassemias or TDT (regular and lifelong transfusions required)
      a. β-thalassemia major (homozygous β^0 thalassemia)
      b. Nondeletional HbH disease

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c. Severe HbE/β-thalassemia
d. Hb Bart's hydrops fetalis syndrome (survived)

Geographic distribution of hemoglobinopathies and thalassemias parallels the distribution of *Plasmodium falciparum*. Heterozygotes with these disorders are relatively resistant to *P. falciparum* malaria.

It is predicted that frequency of inherited hemoglobin disorders is likely to increase because of following factors: (1) natural selection of heterozygotes due to protection afforded against malaria, (2) practice of consanguineous marriages in some affected ethnic groups, (3) increased survival of affected patients due to improvements in social conditions and public health, and (4) rapidly increasing population size in general in India.

Correct diagnosis of hemoglobin disorders is essential for proper management, for genetic counseling of prospective parents, and for prenatal diagnosis and decision regarding termination of pregnancy.

Different inherited disorders of hemoglobin are as follows:

Hemoglobinopathies

Hemoglobins with reduced solubility: Some point mutations in the β-globin gene cause alteration in the solubility of hemoglobin. In these, a nonpolar amino acid is substituted for a polar one near the surface of the molecule. For example, point mutation GAG→GTG at the 6th codon of β-globin gene leads to the formation of sickle hemoglobin (HbS). Upon deoxygenation HbS polymerizes causing formation of sickle cells; these cells are less deformable than normal, cause vascular occlusion and are also phagocytosed in spleen.

Another example is HbC, which is formed by substitution of lysine for glutamic acid at position 6 of globin chain (β^{6}Glu→Lys). Crystallization of HbC increases rigidity of red cells, which are destroyed in spleen.

Unstable hemoglobins: Instability of hemoglobin molecule arises from mutations that interfere with structural relationship between globin chains and heme. Instability results in precipitation of hemoglobin with formation of Heinz bodies that attach to the red cell membrane. The red cells become rigid and are sequestered and destroyed in the spleen. The unstable hemoglobins are also called as congenital Heinz body hemolytic anemias.

Hemoglobins with low oxygen affinity: Some mutations in globin gene stabilize the hemoglobin in deoxygenated state. There is higher than normal oxygen delivery to the tissues so that oxygen demands are met at a lower hemoglobin concentration ("pseudoanemia"). A large percentage of deoxygenated hemoglobin can cause cyanosis.

Hemoglobins with increased oxygen affinity: An example is Hb Chesapeake in which a mutation at $\alpha_1\beta_2$ interface stabilizes the hemoglobin in the oxygenated or relaxed state. Oxygen is not released readily to the tissues resulting in tissue hypoxia and compensatory erythrocytosis.

Hemoglobin M: M hemoglobins arise from mutations that stabilize iron of heme in the nonfunctional ferric state. Such hemoglobins are unable to bind oxygen and produce cyanosis.

Structural hemoglobin variants causing phenotype of thalassemia: Some mutations produce an abnormal hemoglobin molecule as well as reduced synthesis of globin chains, e.g., hemoglobin E (β^{26} Glu→Lys) which is associated with the phenotype of β-thalassemia.

Nomenclature of hemoglobinopathies: More than 700 hemoglobinopathies have been reported so far. The majority of them are clinically insignificant. Variant hemoglobins are denoted by various methods like letters, name of the place where first discovered, residence of the prepositus, or family name of the index case. The first abnormal hemoglobin discovered was

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