Notes

18

HEMOLYTIC ANEMIA

18.1 INTRODUCTION

Hemolytic anaemias are anaemias that result from increased destruction of red cells or due to a shortened life span of red cells

OBJECTIVES

After reading this lesson, you will be able to:

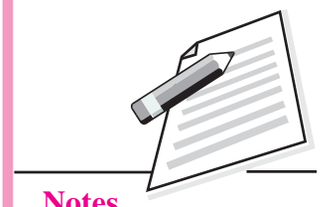
- define hemolytic anaemia, extravascular hemolysis & Intravascular hemolysis
- describe the haematological findings in hemolysis
- explain the biochemical findings in hemolysis
- classify hemolytic anaemia.

18.2 DEFINITION

Hemolytic anemia results from increased destruction of red cells or due to a shortened life span of red cells. They are characterized by increased red cell destruction together with compensatory regeneration.

18.3 PATHOGENESIS

The normal life span of the red cell is about 120 days. When there is hemolysis the bone marrow compensates by increasing erythropoiesis by 6 to 8 times the normal number of RBC produced. Anemia results when the bone marrow is no longer able to compensate the degree of hemolysis.



18.4 CLASSIFICATION OF HEMOLYTIC ANEMIA

Based on the site of red cell destruction, hemolytic anemia is classified as:

(a) Intravascular: when the destruction is predominantly within the circulation

(b) Extravascular: when the destruction is predominantly within tissue macrophages. The macrophages in the spleen, liver bone marrow and lymph nodes phagocytose and destroy red cells.

Hemolytic anemia can also be classified as due to intrinsic (defect in the red cell) or extrinsic (red cell is normal, anemia is secondary to exposure to drugs, infection or other factors) abnormalities.

18.4.1 Defects inside the RBC (Intracorpuscular defects)

These conditions are usually hereditary except paroxysmal nocturnal hemoglobinuria (PNH) which is an acquired condition.

(a) Red cell membrane defects - eg. Hereditary spherocytosis, elliptocytosis

(b) Abnormalities of hemoglobin synthesis

Decreased globin synthesis – quantitative defect eg. Thalassemias

Abnormal globin synthesis – qualitative defect eg. Sickle cell anemia

(c) Abnormalities of red cell enzymes – eg. G6PD deficiency

18.4.2 Defects outside the RBC (Extracorpuscular defects)

The red cells are normal but the life span is shortened because of external factors.

(a) Immune hemolytic anemia- alloimmune, autoimmune, drug induced

(b) Parasites – eg. Malaria

(c) Bacterial – eg. Clostridia

(d) Venoms – eg. Snake venoms

(e) Red cell fragmentation seen in disseminated intravascular coagulation, hemolytic uremic syndrome, march hemoglobinuria, prosthetic heart valves etc.

(f) Drug induced

(g) Burns

18.5 BIOCHEMICAL CHANGES DURING HAEMOLYSIS

When the red cells are destroyed, the hemoglobin is broken down to globin and heme.

(a) Globin chains are broken down to amino acids which are re-used for protein synthesis.

(b) The heme ring is opened up and the iron is released, bound to transferrin, recirculated and used for new RBC formation.

(c) The protoporphyrin ring is opened up and during this process carbon monoxide is released and excreted through expired air.

(d) Biliverdin is formed from the protoporphyrin**.** Biliverdin is converted to un-conjugated (indirect) free bilirubin by biliverdin reductase**.** The unconjugated bilirubin is bound loosely to serum albumin and is conjugated to bilirubin glucuronide (direct or conjugated bilirubin) in the liver.

(e) The conjugated bilirubin is excreted in bile giving it a yellow green colour

(f) Most of the bile secreted in the small intestine is reabsorbed and transported back to the liver. This is called the enterohepatic circulation of bilirubin.

(g) In the intestine the remaining bilirubin is converted to stercobilinogen

(h) Some of the stercobilinogen is reabsorbed and excreted as urobilinogen in urine.

Thus in hemolytic anemia, the serum total bilirubin is mildly increased, most of it is indirect bilirubin, urine urobilinogen and fecal stercobilinogen are increased and there is increased storage iron.

When red cells get destroyed in the blood stream releasing hemoglobin into plasma it is called **intravascular hemolysis**. The hemoglobin binds to a plasma protein called haptoglobin and the Hb-Haptoglobin complex is taken up by tissue macrophages. The hemoglobin is then degraded to biliverdin. When all the haptoglobin is saturated the free hemoglobin is increased resulting in **hemoglobinemia** and is excreted by the kidney as **hemoglobinuria**. Some of this hemoglobin in the glomerular filtrate is reabsorbed by the tubular epithelium and stored as hemosiderin. Demonstration of hemosiderin in urine **(hemosiderinuria)** indicates recent intravascular hemolysis.

INTEXT QUESTIONS 18.1

1. Haemolytic anaemias occurs because of ..... or ..... of red cells
2. Normal life span of red cells is .....

3. Normal mechanism of red cell destruction is .....
4. During hemolysis haemoglobin is broken down as ..... & .....
5. Bilirubin is excreted as ..... in urine
6. Match the following

1. Red cell membrane defect	(a) Thalassemias
2. Abnormal globin synthesis	(b) G6PD deficiency
3. Decreased globin synthesis	(c) Hereditary Sperocytosis
4. Abnormal red cell enzymes	(d) Sickle cell anaemia

18.6 GENERAL LABORATORY FEATURES OF HEMOLYTIC ANEMIA

A. Hemoglobin, PCV and RBC count are reduced.

B. Peripheral blood smear shows variable anemia with anisocytosis, poikilocytosis, polychromasia, fragmented red cells, nucleated RBC and basophilic stippling. These findings are seen in most of the hemolytic anemias. Depending on the underlying cause of hemolysis the more specific findings like sickle cells, spherocytes or target cells may be present.

C. Reticulocyte count is increased.

D. Bone marrow examination has limited role in the diagnosis of hemolytic anemia. The bone marrow shows cellular marrow particles and markedly hypercellular smears. There is marked erythroid hyperplasia with normoblastic maturation resulting in reduction of myeloid to erythroid ratio. Myeloid maturation and thrombopoiesis are normal.

E. Biochemical changes are as described above. Serum LDH is elevated.

F. Red cell life span is decreased.

G. Special investigations have to be done to confirm the diagnosis. These investigations will depend on the underlying cause of hemolysis.

18.7 HEREDITARY SPHEROCYTOSIS

18.7.1 Definition

This is an anemia characterized by the presence of many small red cells or spherocytes in the blood. The abnormal shape of the red cells is due to the lack of one or more of the cytoskeletal proteins (mainly spectrin and ankyrin) of the red cell membrane. The abnormal shape results in lack of flexibility of the cell membrane while passing through small capillaries. The red cells are trapped in the microcirculation and have a decreased life span.

18.7.2 Etiology

This red cell membrane defect is usually an inherited disorder. It is inherited as an autosomal dominant trait and affects both sexes equally.

18.7.3 Clinical Presentation

Young males and females present with history of repeated attacks on mild jaundice, fever, gall stones, leg ulcers and splenomegaly. Rarely when the defect involves more than one protein, there may be neonatal hyperbilirubinemia.

18.7.4 Laboratory Diagnosis

1. Hemoglobin, PCV, RBC count are mildly decreased
2. MCV and MCH are normal, MCHC is increased
3. Peripheral blood smear (figure 4) characteristically shows spherocytes which are well hemoglobinized red cells with a low mean cell diameter. Spherocytes lack the central pallor which is encountered in normal red cells. The number of spherocytes per high power field varies from patient to patient. Poikilocytes and polychromatophils may be seen. Spherocytosis can also be seen in autoimmune hemolytic anemia, ABO hemolytic disease of the newborn and bacterial toxins like Clostridium.
4. WBC and platelet counts are normal
5. **Osmotic fragility test**

   This test demonstrates that the surface area to volume ratio is reduced in the presence of spherocytosis. Normal red cells can withstand hypotonicity and can increase in size by about 70% (because of the biconcave shape) before their membrane is stretched. Spherocytes on the other hand when placed in progressively more hypotonic solutions, because of their round shape, are unable to swell further as water enters the cells and they rupture sooner than normal red cells. Thus spherocytes, whatever the reason for their formation will show increased osmotic fragility. Osmotic fragility is also increased in hereditary elliptocytosis and hereditary stomatocytosis. Iron deficient microcytic hypochromic red cells and thalassemic red cells show decreased osmotic fragility.
6. Direct Coomb's test must be performed to rule immune cause for the formation of spherocytes.
7. Biochemical tests show indirect bilirubinemia, increased excretion of urobilinogen and stercobilinogen and increased serum LDH.

8. Analysis of cytoskeletal proteins to demonstrate the protein responsible may be done by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis)
9. Flow cytometric (dye binding) test

18.8 HEREDITARY ELLIPTOCYTOSIS

Hereditary elliptocytosis syndrome are a group of genetic disorders which are characterized by elliptocytes on peripheral blood smear (figure 5). While most are well compensated hemolytic anemias which are accidently identified during routine peripheral blood examination, some present with moderate to severe hemolytic anemia.

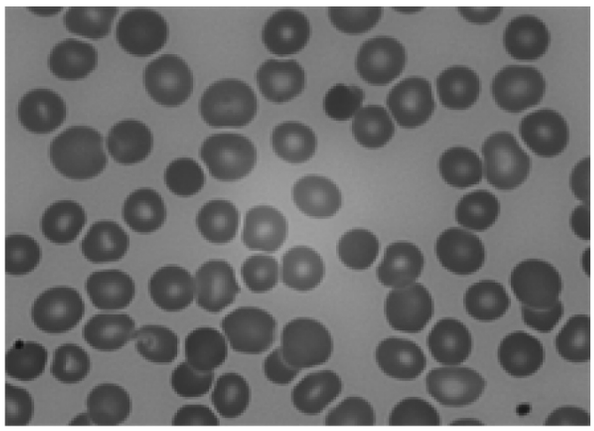


Fig. 18.1: Spherocytes

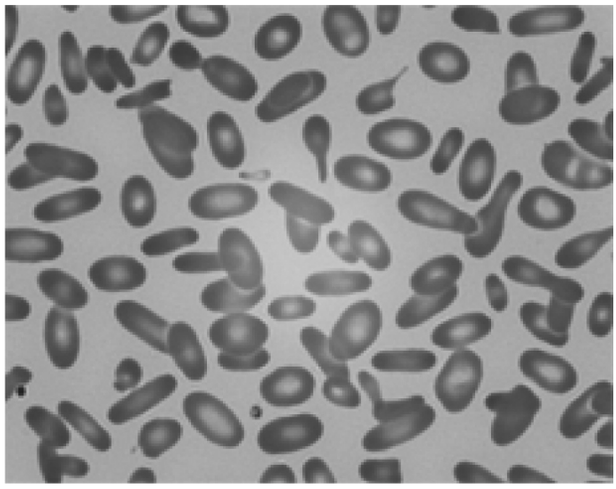


Fig. 18.2: Elliptocytosis

INTEXT QUESTIONS 18.2

1. Hereditary spherocytosis is characterized by presence of ..... in the blood
2. Hereditary sperocytosis is inherited as ..... trait
3. Autosomal dominant inherited disorder of red cell is .....
4. Absence of red cell membrane protein causes .....

18.9 PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

It is an acquired non-malignant clonal disorder where there is expansion of one or more hematopoietic stem cells. It occurs due to mutation in the PIGA gene which leads to production of stem cells which are deficient in glycosylphosphatidylinositol-anchored proteins (GPI-AP). These include complement defense proteins like CD55 (DAF) and CD59; RBC membrane proteins like acetyl choline esterase, etc.

PNH red cells are abnormally sensitive to hemolysis in the presence of complement and a low pH. Hemolysis may vary from mild to massive requiring transfusions and the patient may develop acute tubular necrosis of the kidneys. Classically, the patients have episodic nocturnal intravascular hemolysis and hemoglobinuria. In other patients there may be low grade intravascular hemolysis associated with infection or stress. Associated thrombocytopenia, leucopenia and thrombosis at unusual sites are frequently noted. PNH is often associated with aplastic anemia

Laboratory Diagnosis

1. Mild to severe degree of anemia may be seen.
2. Leucopenia and thrombocytopenia is frequent
3. Reticulocyte count is increased
4. Urine is positive for hemoglobinuria and hemosiderinuria.
5. Biochemical changes seen in other causes of intravascular hemolysis are present.
6. Ham (acidified serum lysis) test: Patient's red cells show abnormal tendency to hemolysis in the presence of a mild acid pH and fresh complement.
7. Direct Coombs test is negative.
8. Flow cytotometry analysis of GPI linked proteins.

WHAT HAVE YOU LEARNT

- Haemolytic anaemia result from increased destruction of red cells or due to shortened life span of red cells
- Normal life span of red cell is 120 days
- Normal mechanism of red cell destruction is called Extravascular haemolysis
- When red cells are destroyed they are broken down to globin and haeme
- Bilirubin is excreted as urobilinogen in urine
- Red cells when destroyed in blood stream, releasing Hb into plasma is called Intravascular haemolysis
- Haemolytic anaemia is classified based on causes as Defects inside RBCs and defects outside RBC
- Lack of cytoskeletal proteins of red cell membrane causes abnormal shape of red cell causing Hereditary Spherocytosis and is characterized by presence of Spherocytes
- Hereditary Spherocytosis is inherited as autosomal dominant inherited disorder
- Hereditary Ellipatocytosis is an autosomal dominant inherited disorder
- Absence of two or more red cell membrane protein causes Hereditary Pyropoikilocytosis

TERMINAL QUESTIONS

Write short notes on

1. Spherocytosis
2. Osmotic fragility test
3. Biochemical findings in Hereditary spherocytosis
4. PNH

ANSWERS TO INTEXT QUESTIONS

18.1

1. Increased destruction, shortened life span

Notes

2. 120 days
3. Extravascular hemolysis
4. Globin & Haeme
5. Urobilinogen
6. 1. (c)
   2. (d)
   3. (a)
   4. (b)

18.2

1. Spherocytes
2. Autosomal dominant
3. Hereditary Elliprocytosis
4. Hereditary Pyropoikilcytosis