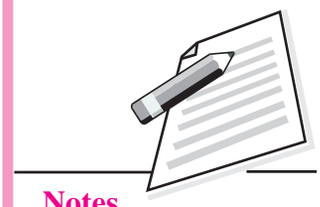

19

HEMOLYTIC ANEMIA DUE TO ABNORMAL HEMOGLOBIN SYNTHESIS

19.1 INTRODUCTION

There are two main mechanisms by which anaemia is produced

(a) **Thalassemia:** A group of disorders characterized by quantitative defect in the synthesis of one or more globin chains. HbA or adult hemoglobin constitutes the maximum amount of red cell hemoglobin and is composed of two alpha and two beta chains.

When alpha (α) globin chains are not produced in normal numbers the condition is alpha (α) thalassemia.

When beta (β) globin chains are not produced in normal numbers the condition is beta (β) thalassemia

(b) **Hemoglobinopathies:** In these conditions there are structural abnormalities in the globin chains leading to qualitative defects. The globin chain is abnormal in structure due to one or more amino acid substitution or deletions or additions. The globin chain so formed is functionally abnormal and the red cell life span is shortened

OBJECTIVES

After reading this lesson, you will be able to:

- describe pathogenesis, lab diagnosis of thalassemia major and minor
- describe pathogenesis of haemoglobinopathies.

19.2 β THALASSEMIA

19.2.1 Inheritance and Pathogenesis

This is an inherited autosomal recessive disorder where there is a decrease in the number of beta globin chains being produced. In the heterozygous condition where the patient has one normal gene and the other thalassemia gene, the globin chain imbalance is compensated by an increase in delta chain production and an increase in HbA2. Thus patients who have one beta thalassemia gene are referred to as beta thalassemia trait and have a mild well compensated microcytic hypochromic anemia. This condition is called beta thalassemia minor.

When a person inherits one beta thalassemia gene from each parent he/she has homozygous beta thalassemia and is unable to produce normal HbA. The chain imbalance is so great as to produce moderate to severe anemia. This condition is called beta thalassemia Major or Cooley's anemia. The patient develops a life long transfusion dependent anemia.

19.2.2 Incidence in India

The thalassemia gene is present in the Indian population. It is more common in certain communities like Punjabis, Gujratis, Marwaris and Sindhis.

19.3 β THALASSEMIA MAJOR

19.3.1 Clinical Presentation

The patient usually presents by the age of 5 – 6 months after birth with pallor, frequent attacks of infections and hepatosplenomegaly. The bone marrow tries to compensate for the anemia by increased production of red cells. However these red cells are defective and undergo lysis – a term used for this situation is "ineffective erythropoiesis". The marrow cavity enlarges, the cortex of the bone thins and many skeletal abnormalities result due to this. Iron overload results in deposition and damage of organs like heart, liver and pancreas. The patient ultimately dies from frequent infections, resultant anemia and iron overload from repeated transfusions.

19.3.2 Laboratory Diagnosis

1. Hemoglobin, PCV, RBC count all decreased. Hb may be 3.0 – 4.0 g/dL
2. MCV, MCH and MCHC decreased. RDW is markedly increased.
3. WBC – Leukocytosis may be present. Many nucleated RBC are present, falsely elevating the WBC count.

4. Platelet count is normal. May be decreased if there is pooling in the spleen.
5. Peripheral blood smear (Fig. 19.1) shows moderate to severe anemia with marked anisocytosis, poikilocytosis, microcytosis, fragmented red cells and polychromasia. Many nRBC, red cell inclusions and basophilic stippling and target cells are also seen.

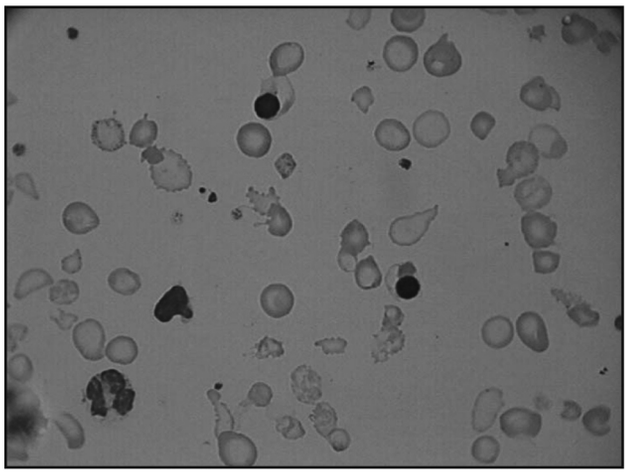

Fig. 19.1: Beta Thalassemia Major

6. Reticulocyte count is increased.
7. Biochemical tests show mild indirect bilirubinemia, increased excretion of urobilinogen and stercobilinogen, increased serum LDH and serum ferritin.
8. Hemoglobin electrophoresis using agarose gel at a pH of 8.6 or citrate agar electrophoresis at pH6.0 can be used to see the electrophoretic pattern of the hemoglobin and detects the common clinically significant hemoglobin variants. Isoelectric focusing is an alternative method.
9. The HbF may be quantified by a test called the Alkali Denaturation test
10. Hemoglobin analysis using High Performance Liquid Chromatography (HPLC) has largely replaced the more time consuming techniques described above. The various hemoglobins may be quantified using the HPLC method. The patient's hemoglobin in beta thalassemia major is mainly HbF with normal amounts of HbA2. In severe β^0 thalassemia there will be no detectable HbA, in β^+ thalassemia small amounts of HbA may be present.
11. Globin chain gene analysis can be done to identify various DNA mutations.
12. Detailed family studies, DNA analysis and screening of all blood relatives of the index case should be undertaken to identify carriers.
13. Genetic counseling should be given to prevent the birth of children with thalassemia.

19.3.3 Treatment

1. Blood transfusions to maintain Hb 10.5 to 11.0g/dL
2. Chelation of iron
3. Bone marrow transplantation

19.4 β THALASSEMIA MINOR

19.4.1 Definition

This is an inherited defect in haemoglobin synthesis where there is a decrease in globin chain synthesis. When the patient inherits two thalassemia genes, one from each parent, he/she develops a severe form of anaemia called thalassemia major. A person who has one thalassemia gene has a mild hypochromic microcytic anaemia which must be differentiated from iron deficiency anaemia.

19.4.2 Laboratory Diagnosis

(a) Haemoglobin, PCV, mildly decreased. **RBC count is increased.**

(b) MCV, MCH are decreased but MCHC is normal. RDW is mildly increased ~14 – 15%

(c) Blood film shows mild anaemia, uniformly microcytic hypochromic red cells with no polychromasia. Target cells may be present. WBC and platelets are normal. Fig. 19.2

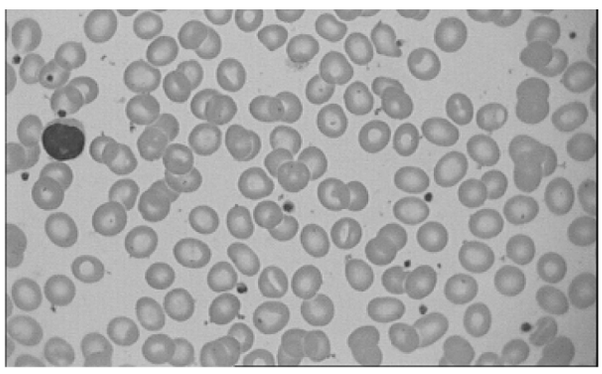

Fig. 19.2: Beta Thalassemia Minor

(d) Reticulocyte count is normal.

(e) Bone marrow is normal with normal iron stores

(f) Biochemical tests show normal serum iron, normal serum TIBC and ferritin

(g) The diagnosis is made by performing a haemoglobin analysis by HPLC (high performance liquid chromatography). Patients with beta thalassemia trait show the presence of raised HbA_2 in the range of 3.7- 7.0% with 80′ – 85% HbA and < 1.0%HbF

IMPORTANT

In the Indian population where the incidence of the beta thalassemia gene is 3- 15% depending on the region, it is important to detect the thalassemia carrier and offer genetic counseling and screening of the spouse to prevent the birth of children with beta thalassemia major.

19.5 HAEMOGLOBINOPATHIES

19.5.1 Definition

In these conditions there are structural abnormalities in the globin chains leading to qualitative defects in the hemoglobin. The globin chain is abnormal in structure due to one or more amino acid substitution, deletions or additions. The globin chain so formed is functionally abnormal and the red cell life span is shortened. There are numerous hemo-globinopathies described but most of them do not produce clinical disease.

19.5.2 Important Haemoglobinopathies

19.5.2.1 Sickle Haemoglobin

Sickle cell hemoglobin (HbS) is caused by a single amino acid substitution in the β globin chain. The sixth amino acid glutamic acid is replaced by valine (HbS $\alpha 2\beta 2^{6glu\text{-}val}$). The abnormal hemoglobin so formed tends to polarize under decreased oxygen tension and deforms the red cells into sickle shaped cells. These cells do not flow freely in the microcirculation. The result is stasis, hypoxic damage to distal tissues and hemolysis.

19.5.2.2 Geographic Distribution

Sickle cell anemia is found in Central and West Africa, the Middle East and along the old slave routes. In India HbS is found in the tribal belts around central India, Maharashtra, Madhya Pradesh, Bihar, Orissa, West Bengal, along the Western Ghats, Tamil Nadu and Kerala.

19.5.2.3 Inheritance

The condition is inherited as an autosomal recessive trait. When a child inherits two HbS genes, one from each parent he/she develops HbS anemia. When a child

inherits one HbS gene from either parent, he/she is called a HbS trait and is asymptomatic. A child may also inherit the HbS gene from one parent and another β globin gene abnormality from the other parent – the so called double heterozygous state eg HbS/bthal or HbS/HbD or HbS/ Hb C etc.

19.5.2.4 Clinical Presentation

The patient with HbS anemia (HbSS) suffers from jaundice, anemia, splenomegaly and frequent infections. Acute events like vaso-occlusive events, bone pains and CNS effects are common. Sequestration of irreversibly sickled cells in lungs, liver, spleen etc can lead to death at a young age.

19.5.2.5 Laboratory Diagnosis

1. Hemoglobin, PCV, RBC count are decreased.
2. MCV, MCH may be normal or decreased, MCHC is normal, RDW is increased.
3 Reticulocyte count is increased.
4. WBC count and platelets are usually increased.
5. Peripheral blood smear (Fig. 19.3) show variable anemia with anisocytosis, poikilocytosis, sickle shaped cells, target cells, polychromasia, nRBC and basophilic stippling. Howell Jolly bodies may be numerous in post splenectomy smears.

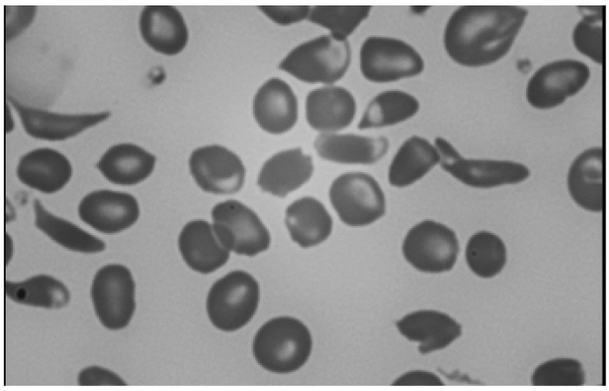

Fig. 19.3: Sickle cells

6. **Sickle cell preparation**

Principle: This is a simple test to demonstrate the decrease solubility and hence the ability of the cells containing HbS to form sickle cells in the absence of oxygen.

Procedure: Make a working solution of disodium hydrogen phosphate and sodium dithionite. Add 5 drops of this freshly prepared reagent to a drop of blood sample anticoagulated in EDTA on a clean glass slide. Place a cover slip over the diluted blood. Seal the edges of the coverslip with paraffin or nail polish to prevent air entry under the coverslip.

Result: Sickling takes place almost immediately in sickle cell anemia and within one hour in sickle cell trait (Fig. 19.4). It is observed at 40x with the condenser of the microscope lowered down.

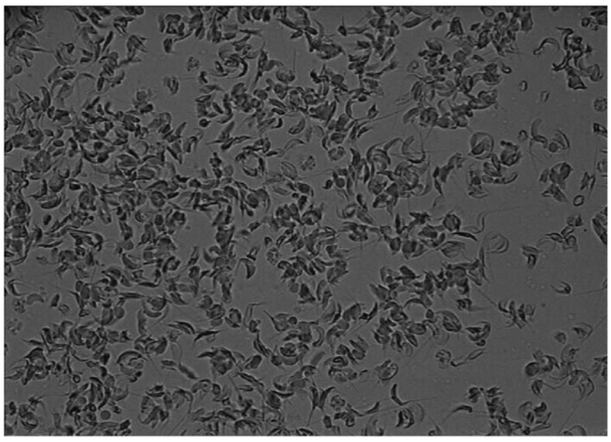

Fig. 19.4: Sickle cell preparation

7. **Solubility test for HbS**

Principle: Hb S is precipitated by high molarity phosphate buffer which increase the turbidity of the solution.

Procedure: The reagent contains KH_2PO_4, K2HPO_4, saponin and sodium dithionite in distilled water. Take 2mL of reagent in a test tube and add 10 µL of packed cells of the patient to it, mix and leave for 5 minutes at room temperature. The solution is now light pink or red in colour. Centrifuge at 1200 rpm for 5 minutes. Result: Observe the contents of the tube by holding the tube 2.5 cm in front of a white card with narrow black lines. The results are compared with appropriately put positive and negative controls.

(a) HbS/S – clear supernatant + curdy precipitate

(b) HbS/A – red supernatant + curdy precipitate

(c) HbA – clear solution, no precipitate

8. Hemoglobin electrophoresis at pH 8.6 on agarose gel or cellulose acetate (Fig. 19.5).

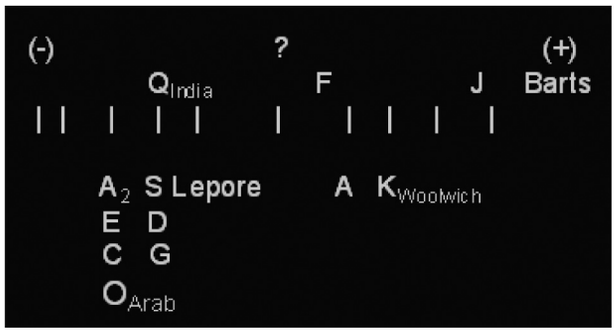

Fig. 19.5: Diagrammatic representation of separation of haemoglobins by electrophoresis

9. Hb S may be quantified using HPLC to separate the abnormal hemoglobin.
10. Biochemical tests show mild indirect bilirubinemia, increased excretion of urobilinogen and stercobilinogen and increased serum LDH.
11. Detailed family studies, DNA analysis and screening of all blood relatives of the index case should be undertaken to identify carriers.
12. Genetic counseling should be given to prevent the birth of children with sickle cell disease.

19.6 OTHER HAEMOGLOBINOPATHIES SEEN IN INDIA

19.6.1 Haemoglobin E

This abnormal hemoglobin is commonly seen in the North Eastern states of India. The abnormal hemoglobin is HbE $\alpha 2\beta 2^{26glu\text{-}lysine}$. It may present as homozygous HbE (HbE/E) or HbE trait (HbE/A) or HbE/β thal. The homogygous HbE and HbE trait are usually asymptomatic but HbE/β thal presents as a moderately severe hemolytic anemia.

19.6.2 Haemoglobin D

This hemoglobin is commonly seen in the Punjabi population. Hb D is $\alpha 2\beta 2^{26glu\text{-}lysine.}$ May present as homozygous HbD (HbD/D) or HbD trait (HbD/A) or HbD/β thal which is a moderately severe hemolytic anemia.

19.6.3 Haemoglobin C

This abnormal hemoglobin is found mainly in West Africa. HbC is $\alpha 2\beta 2^{26glu\text{-}lysine.}$ The heterozygotes are asymptomatic whereas the homozygous individuals develop a mid degree of hemolytic anemia. Significant numbers of target cells are seen in the peripheral blood smear (Fig. 19.6).

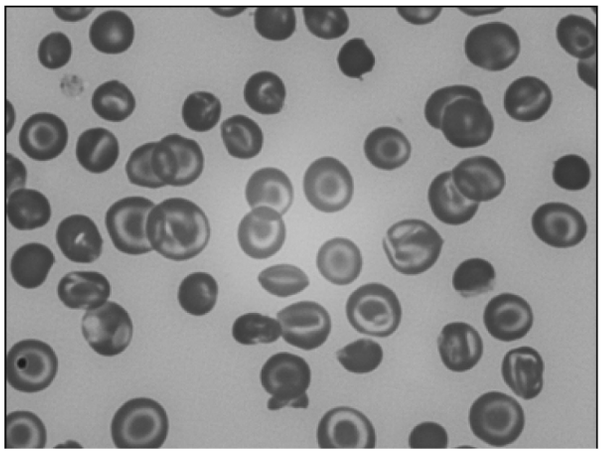

Fig. 19.6: Haemoglobin C

INTEXT QUESTIONS 19.1

1. β thalassemia is inherited as ..... disorder
2. Thalassemia minor causes ..... anaemia
3. Cooley's anaemia is also called as .....
4. Defective red cells undergoing lysis is described as .....
5. Single amino acid substitution in ß globin chain causes ..... anaemia
6. Sickle cell anaemia is inherited as ..... disorder

WHAT HAVE YOU LEARNT

- Decreased production of one or more normal globin chains causes thalassemia. Reduced Alpha (α) globin chains causes Alpha thalassemia and reduced beta (β) globin causes beta thalassemia
- Structural abnormalities in globin chain causes haemoglobinopathies
- β thalassemia is inherited as autosomal recessive disorder
- Heterozygous gene causes mild form of disorder called thalassemia minor
- Homozygous gene causes severe form of disorder called thalassemia major which is also known as cooley's anaemia
- Defective red cells undergoing lysis is described as ineffective erythropoiesis
- Haemoglobin electrophoresis using agarose gel shows mainly of HbF with normal amount of HbA2. In sever thalassemia there will be not detectable

Hb, in β+ thalassemia small amount of HbA may be present. No abnormal Hb are detectable

- HbF may be quantified by Alkali Denaturation test
- Structural abnormalities by substitution or deletion or addition of amino acid in globin chain leading to qualitiative defects
- Sickle Hb is caused by single amino acid substitution in β globin chain. The sixth amino acid glutamic acid is replaced by valine
- Solubility test is used for identifying sickle Hb.

TERMINAL QUESTIONS

Write short notes on

1. Tests to demonstrate sickle haemoglobin
2. Thalassemia minor
3. Laboratory diagnosis of Thalassemia major

ANSWERS TO INTEXT QUESTIONS

19.1

1. Autosomal recessive
2. Hypochromic microcytic anaemia
3. Thalassemia major
4. Ineffective erythropoiesis
5. Sickle cell
6. Autosomal recessive trait