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HEMOLYTIC ANEMIAS DUE TO ABNORMAL RED CELL ENZYMES

20.1 INTRODUCTION

The main metabolic substrate for the RBCs is glucose. It is metabolized by two pathways: approximately 90% of the glucose is metabolized through the Embden Meyerhoff (glycolytic) pathway and the rest by the hexose monophosphate (HMP) pathway.

In the Embden Meyerhoff (glycolytic) pathway glucose is metabolized to lactate through a series of enzymatic steps. Each molecule of glucose gives rise to 2 molecules of ATP. The ATP provides energy to maintain red cell volume, shape and flexibility. An ATP dependent pump in the red cell membrane actively keeps sodium out of the cell and potassium inside. The red cell has the enzymes that are needed for the glycolytic pathway. These enzymes help break down glucose to generate ATP which is the source of energy.

About 10% of the glucose is diverted to the Hexose Monophosphate shunt pathway and this is essential for protection of red cells from oxidative stress. This pathway is necessary for the generation of NADPH which then reduces oxidized glutathione (GSSG) to reduced glutathione (GSH). GSH prevents the accumulation of H_2O_2 and the oxidation of hemoglobin to methemoglobin. When the level of GSH falls, H_2O_2 accumulates in the cell and oxidizes the hemoglobin to methemoglobin which becomes denatured and precipitates as Heinz bodies. These inclusions are rigid and attached to the red cell membrane and make the red cell susceptible to hemolysis. The NADPH required in this pathway is generated by the enzyme Glucose 6 phosphate dehydrogenase (G6PD).

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Any of the enzymes in these pathways may be deficient or dysfunctional and result in hemolysis. The most common enzyme deficiencies are G6PD deficiency and pyruvate kinase deficiency.



OBJECTIVES

After reading this lesson, you will be able to:

- describe G6PD deficiency disorder, its clinical presentation and Laboratory diagnosis
- describe extra-corporeal causes of hemolysis
- explain haemolytic anemia due to red cell fragmentation

20.2 G6PD DEFICIENCY

Glucose 6 phosphate dehydrogenase (G6PD) is responsible for sending glucose 6 phosphate (G6PO₄) into the HMP shunt and the generation of NADPH. Deficiency of G6PD enzyme results in red cells which are unable to degrade oxidant drugs and chemicals because the GSH is not being generated in these cells. On exposure to oxidant stress the deficient red cells form numerous Heinz bodies and undergo hemolysis. The disorder is a X linked recessive disorder. The patients with G6PD deficiency are further classified into variants (Class I to V) based on the magnitude of enzyme deficiency and severity of hemolysis.

20.2.1 Clinical Presentation

The clinical presentation depends on the variant of the G6PD deficiency. Most of the patients are asymptomatic under normal circumstances. However, these patients when exposed to oxidant stress (drugs, chemicals, infections, fever, fava beans) develop acute hemolytic anemia with passage of cola coloured urine, jaundice and in severe cases may develop renal shutdown.

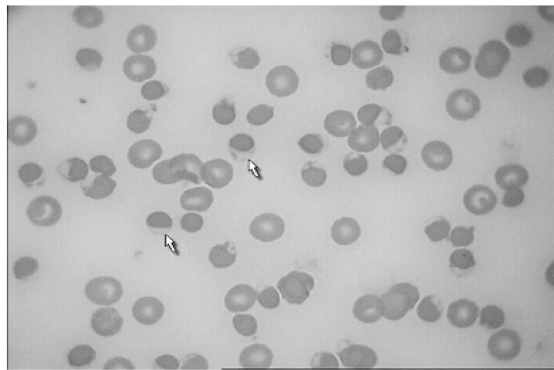
20.2.2 Laboratory Diagnosis

1. Anemia develops secondary to the exposure of oxidative stress. The hematological features are those of hemolytic anemia and are not specific for G6PD deficiency.
2. Peripheral blood smear shows moderate to severe anemia with anisocytosis, poikilocytosis, polychromasia, irregularly contracted red cells, bite cells, spherocytes and nRBC. Bit cells or blister cells (Fig. 20.1) are red cells in

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which the hemoglobin has been pushed to one side leaving an empty bleb or blisters.

3. Heinz body can be demonstrated by brilliant cresyl blue supravital staining.
4. Reticulocyte count is increased
5. Methemoglobin reduction test is a screening test for G6PD enzyme deficiency. It is based on the ability of the test red cells to generate NADPH and convert methemoglobin to hemoglobin. It must be remembered the reticulocytes contain trace amounts of the enzyme even in deficient cells and may give a false positive qualitative result during acute hemolysis
6. G6PD enzyme assays are available for quantifying the enzyme



Arrows indicate "blister cells"

Fig. 20.1: Oxidant haemolysis in G6PD deficiency



INTEXT QUESTIONS 20.1

1. Enzyme deficiencies like & cause haemolytic anemias
2. Cola coloured urine occurs in deficiency
3. G6PD deficiency affects & are carriers
4. test is used for quantifying the enzymes

20.3 EXTRACORPUSCULAR CAUSES OF HAEMOLYSIS

These anemias are caused by factors external to the red cell which is often an innocent bystander. The most important conditions are considered below

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20.3.1 Immune Hemolytic Anemias

1. Pathogenesis

Immune hemolytic anemias are due to antibody production against the red cells. The antigen(s) are present on the red cell membrane and the antibody in the serum binds to the antigen to form an antigen – antibody complex on the cell surface. This complex is removed by macrophages in the liver and spleen (Extravascular hemolysis). During this process the red cell membrane is lost and microspherocyte form.

2. Types of Immune Hemolytic Anemia.

A. Alloimmune Hemolytic Anemia

This type of anemia occurs in two situations:

- (a) Mismatched blood transfusion when blood of the wrong ABO group is transfused to a patient. The patient's serum contains naturally occurring IgM isoantibodies (anti A and/or anti B). The antibody binds to the transfused cells and produces intravascular hemolysis of the transfused red cells.
- (b) Hemolytic disease of the new born which occurs during pregnancy when Rh negative mother has a Rh positive baby.

B. Autoimmune Hemolytic Anemia

In this condition the antibody is produced by the body against its own red cells. Based on the temperature at which these antibodies act, they can be classified as warm acting or cold active or a mixture.

Cold active antibodies donot attach to RBCs at room temperature, but their affinity increases as the temperature approaches 0°C. They are usually IgM type and fix complement. In contrast to this warm active antibodies act at 37°C, are IgG type and do not fix complement.

Autoantibodies may be idiopathic or secondary to other autoimmune disorders like systemic lupus erythematosus, lymphoproliferative disorders, infections like syphilis and drugs. Hemolysis in AIHA can be either intravascular or extravascular.

Drug Induced Immune Haemolytic Anemia

Drugs may cause an antibody mediated haemolytic anemia by different mechanisms. The anemia disappears when the drug is withdrawn.



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3. Laboratory Diagnosis of Immune Hemolytic Anemias

- A. Haemoglobin, PCV, RBC count is decreased.
- B. In cold active AIHA, MCV may be artifactually elevated, producing a falsely elevated MCHC (due to red cell agglutination)
- C. Reticulocyte count is elevated
- D. Peripheral blood smear shows variable anemia. Agglutination of red cells may be present when cold antibodies are present. Spherocytes are seen in warm type AIHA
- E. Biochemical tests show raised indirect bilirubin and LDH. Intravascular hemolysis presents with hemoglobinuria, hemosiderinuria and decreased serum haptoglobin
- F. Serological tests: The diagnosis of AIHA is established by detection of antibodies and or complement on the surface of the patient's red cells. In Direct Coomb's test patient's red cells are mixed with sera containing antibodies. In Indirect Coomb's test, the patient's serum is tested against reagent red cells.



INTEXT QUESTIONS 20.2

- 1. Iso immune haemolytic anemia occurs because of
- 2. Alloimmune haemolytic anemia in new born due to
- 3. In autoimmune haemolysis anemia test is positive
- 4. In alloimmune haemolytic anemia test is positive

20.4 HAEMOLYTIC ANEMIA DUE TO RED CELL FRAGMENTATION

Occasionally an intravascular hemolytic process occurs when red cells are broken secondary to excessive physical trauma. This may occur inside blood vessels which are blocked because of narrowing or fibrin deposition. RBC may also be broken if there is mechanical obstruction like abnormal or synthetic heart valves. Turbulence in blood flow may also cause red cell fragmentation:

Causes of red cell fragmentation

- A. Abnormalities of heart and large vessels
 - (a) Mechanical heart valves

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- (b) Renal artery stenosis
- (c) Large hemangioma
- B. Small vessel disease (microangiopathic hemolytic anemia)
 - (a) Hemolytic uremic syndrome (HUS)
 - (b) Thrombotic thrombocytopenic purpura (TTP)
- C. Disseminated intravascular coagulation (DIC)
- D. Lupus erythematosus

Laboratory Diagnosis

1. The blood findings depend on the degree of hemolysis. If the hemolysis is compensated, the Hb, red cell count and indices are near normal
2. Peripheral blood smear (Fig. 20.2) shows variable anemia, anisocytosis, poikilocytosis, fragmented red cells, polychromasia, nRBC and red cell inclusions.
3. Biochemical test show raised indirect bilirubin and LDH. Serum haptoglobin is reduced.
4. Urine may show hemoglobinuria or hemosiderinuria.

The term Microangiopathic Haemolytic Anemia may also be used for red cell fragmentation occurring in association with small vessel disease.

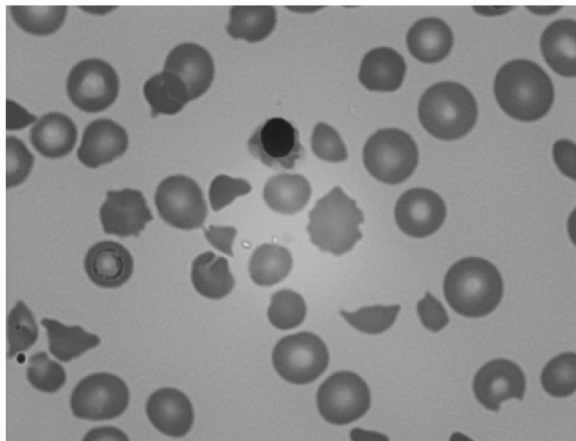


Fig. 20.2: Red cell Fragmentation in DIC



WHAT HAVE YOU LEANT

- Red cells has many enzymes that are needed for glycolytic pathway

Hemolytic Anemias Due to Abnormal Red Cell Enzymes

- These enzymes help breakdown glucose to generate ATP which is the source of energy
- Any of the enzymes in the pathway may be deficient or dysfunctional and result in hemolysis
- Common enzyme deficiency are G6PD deficiency and Pyruvate kinase deficiency
- G6PD is responsible for sending G6PO4 into HMP shunt and generation of NADPH
- Deficiency of G6PD enzyme makes red cells unable to degrade oxidant drugs and chemicals as GSH is not generated in cells
- On exposure to oxidant stress, the red cells form Heinz bodies and undergo vascular hemolysis
- Immune hemolytic anemias are due to antibody production against red cells
- During this process red cell membrane is lost and destroyed by hemolysis
- Isoimmune hemolysis occurs because of mismatch blood transfusion causing intra vascular hemolysis
- Alloimmune hemolysis occurs as hemolytic disease of newborn due to anti D
- Autoimmune hemolytic anemia occurs because of idiopathic causes or because of other auto immune disorder like Systemic Lupus Erythematosis
- Hemolytic process occurs when red cells are broken inside small blood vessels because of narrowing or fibrin deposition



TERMINAL QUESTIONS

Write short notes on the following

1. Oxidant haemolysis in G6PD deficiency
2. Haemoglobinuria and haemosiderinuria
3. Autoimmune Hemolytic anemia
4. Microangiopathic hemolysis
5. Coomb's tests – Direct and Indirect
6. Types of Immune hemolytic anemia

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ANSWERS TO INTEXT QUESTIONS

20.1

1. G6PD & Pyruvate kinase
2. G6PD deficiency
3. Males & Females
4. G6PD enzyme assays

20.2

1. Mismatched blood transfusion
2. Anti D
3. Direct coomb's test
4. Indirect coomb's test