Notes

11

FORMATION OF PLATELETS AND THROMBOCYTOPENIA

11.1 INTRODUCTION

Platelets are small 2μm in diameter with a volume of 8fl. They are anucleate fragments which on Romanowsky stained smears are seen in clumps with occasional reddish granules. They show variation in size and shape. Platelets play an important role in coagulation.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the steps in formation of platelets
- explain the causes of thrombocytopenia

11.2 FORMATION OF PLATELETS

Platelet formation begins in the yolk sac and then shifts to the liver and finally the marrow. Platelets are formed from cells in the bone marrow called as **megakaryocytes** under the influence of thrombopoietin.

The first morphologically recognizable megakaryocyte is called a **megakaryoblast or stage 1 megakaryocyte** which is 6-24 μm in size with a relatively large nucleus, loose chromatin, multiple nucleoli and scant basophilic cytoplasm. It comprises of 20% of all megakaryocytes in the normal bone marrow.

This gives rise to the **stage II megakaryocyte** which is 14-30 μm in diameter,and has a lobulated nucleus and more abundant but less intensely basophilic cytoplasm. It comprises 25% of marrow megakaryocytes.

Mature megakaryocytes or stageIII/IV megakaryocytes are very large cells, 40-60 µm in size with eccentrically placed highly lobulated single nucleus. The cytoplasmic basophilia is further reduced. Stage 4 megakaryocytes comprise of 50% of marrow megakaryocytes and are wholly engaged in platelet formation.

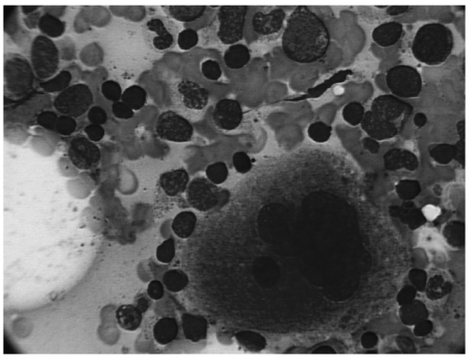


Fig. 11.1: A stage IV megakaryocyte in the bone marrow

Platelets are formed by fragmentation of the cytoplasm of megakaryocyte. It has been shown that each megakaryocyte can give rise to 1000 to 5000 platelets. Each day an adult human produces 1×10^{11} platelets. In times of increased demand platelet production can rise more than 10 times. The normal platelet count is $1.5\text{-}4\times10^{9}$/L. For any individual the count remains within a fairly narrow range.

Platelets are tiny anucleate structures 7.5 to 10.5 fl in size. On peripheral smear they appear as tiny blue gray structures with reddish granules. Platelets released from marrow under conditions of stress e.g. in thrombocytopenia are larger and often beaded in shape. These are termed as **stress platelets**, whereas the normal young platelets recently released from the marrow are called **reticulated platelets**. Platelets have a life span of 8-12 days after which they are removed by the spleen.

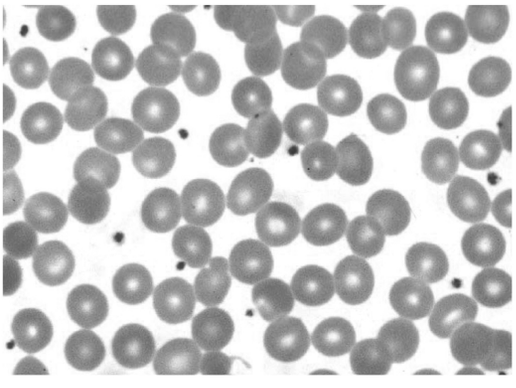


Fig. 11.2: Platelets as seen on peripheral smear

INTEXT QUESTIONS 11.1

1. Platelets are anucleate fragments which are ..... in size.
2. Platelets plays an important role in .....
3. Platelets which are formed from the bone marrow cells under the influence of thromboprotein are called as .....
4. Fragmentation of cytoplasm of megakaryocyte results in the formation of .....
5. ..... are nothing but the platelets released from marrow under stress.

11.3 STRUCTURE OF PLATELETS

The plasma membrane of platelets consists of phospholipids, glycolipids and several surface receptors such as glycoprotein IIb-IIIa which mediate interaction of platelets and other cells as required for normal function of platelets. Platelets possess secretory granules: α granules(PF4,vWF,PDGF,VEGF, β thromboglobulin) and dense bodies (ADP ,Ca, serotonin and ATP). The contents are released when the platelet is activated and they facilitate the coagulation process. Platelets exist in two distinct forms: resting and activated. The activated state results from stimulation by agonists such as thrombin.

Function of platelets

Platelets play a central role in normal hemostasis. They participate in primary hemostasis by adhering to sites of vascular injury and forming a primary hemostatic plug. After vascular injury, platelets come in contact with subendothelial collagen. On contact with the collagen, platelets undergo the following changes: they adhere to subendothelial collagen via vWF which is a bridge between platelet surface gpIb and the exposed collagen. This interaction stabilizes the platelet adhesion against the shear forces of flowing blood. It's importance is highlighted by the fact that deficiency of von Willebrand factor (von Willebrand disease) or gp Ib(Bernard Soulier syndrome) results in a bleeding tendency due to lack of primary hemostasis. The platelets undergo shape change and then there is release of the granule content. ADP is the most important content which causes platelet aggregation and augments release of ADP from other platelets.

After activation of platelets there is expression of a phospholipids complex on the platelet surface which provides a critical nucleation site for calcium and factor binding which then initiates the coagulation pathway leading to formation of thrombin. Platelets enhance contact activation and help in generation of tissue

factor thus further promoting coagulation and leading to fibrin formation. Thrombin results in further aggregation and with fibrin stabilizes the aggregated platelets creating a secondary hemostatic plug.

Causes of thrombocytopenia

A fall in platelet count below the normal level is called thrombocytopenia. It is the most common cause of abnormal bleeding. The causes are tabulated below.

A decreased platelet count (thrombocytopenia) can result from a marrow production problem or a peripheral platelet destructive process. Bleeding complications or even death can result in the presence of a severely decreased platelet count.

INTEXT QUESTIONS 11.2

Match the following

1. Decreased platelet content	(a) Von Willebrand disease
2. Two forms of platelet	(b) TTP, DIC
3. Hemostasis	(c) Thrombocytopenia
4. Lack of primary hemostasis	(d) Resting, Activated
5. Non immune cause of thrombocytopenia	(e) Platelet

Table 11.1 Causes of thrombocytopenia

I. Deficient platelet production

- Hypolasia of megakaryocytes due to drugs, irradiation and aplasia of bone marrow
- Defective thrombopoiesis

II. Accelerated platelet destruction(most common)

A. Immune causes

(a) Autoimmune

- idiopathic

Secondary infections, pregnancy, collagen

- infections, collagen vascular diseases, drugs, pregnancy, miscellaneous

(b) Alloimmune

- neonatal thrombocytopenia
- posttransfusion purpura

B. Non immune

- TTP, DIC
- platelet damage by abnormal vascular surfaces

III. Abnormal pooling of platelets

- disorders of spleen
- massive blood transfusion

Platelet count

The platelet count is one of several laboratory assays of importance in the functional evaluation of the hemostatic system.

Platelet count can be done manually. In most laboratories it is measured by automated hematology analysers which provide more accurate counts.

Equipment required

Neubauer chamber

Reagents required

1%ammonium oxalate

Sample

Venous blood collected in EDTA

Method

1. Make a 1 in 20 dilution of blood by mixing 0.2ml of blood with 3.8ml of diluent.Mix well.
2. Fill a Neubauer chamber with the mixture using a Pasteur pipette.
3. Place the chamber in a moist petri dish and leave undisturbed for 20min to allow the platelets to settle down.
4. Examine under the low power with the condenser kept down.The platelets appear as refractile particles.
5. Count the number of platelets in the RBC counting area in the four peripheral squares and the central square.

   Platelet count = N × 1000/mm^3

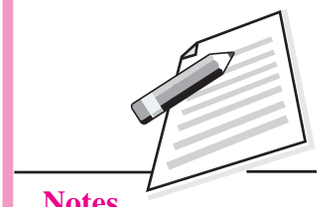
Normal platelet count is 150-400×10^9/L

INTEXT QUESTIONS 11.3

1. The commonest cause of thrombocytopenia is

   (a) increased pooling (b) decreased production

   (c) increased destruction (d) any of the above



2. The granule content which causes platelet aggregation is
   (a) ADP (b) ATP
   (c) Serotonin (d) All of the above
3. What is the normal platelet count

WHAT HAVE YOU LEARNT

- Platelets are tiny anucleate structures 7.5 to 10.5 fl in size. They are formed by cytoplasmic fragmentation of cells in the bone marrow called megakaryocytes. On peripheral smear they appear as tiny blue gray structures with reddish granules. They have a life span of 10 days after which they are removed by the spleen. Platelets play an important role in coagulation. They adhere to subendothelial collagen at sites of injury, release the contents of their granules and aggregate to form a primary hemostatic plug. They also provide a surface for interaction of coagulation factors.
- Thrombocytopenia refers to a decrease in the number of circulating platelets. It results from deficient production, increased destruction and abnormal pooling of platelets.

TERMINAL QUESTIONS

1. Explain the formation of platelets
2. What are the causes of thrombocytopenia

ANSWERS TO INTEXT QUESTIONS

11.1

1. 7.5 – 10.5 fl
2. Coagulation
3. Megakaryocytes
4. Platelets
5. Stress platelets

11.2

1. c 2. d 3. e 4. a 5. b

11.3

1. c 2. a 3. 1.5-4 × 10^9/L