

B.N. Bandodkar College of Science, Thane

T.Y. B.Sc – Paper II

Haematology

Blood Coagulation / Blood clotting

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**Introduction:**

When blood is shed, it loses its fluidity within few minutes and sets into a semisolid jelly called clot. This phenomenon of formation of clot is called as coagulation or clotting of blood. The clot gradually retracts and a fluid separates out, called serum.

Blood coagulation is a property of plasma, RBC, WBC, do not directly take part in clotting process but get caught up in the meshes of the clot.

Blood platelets play important role in coagulation.

**Significance :**

the phenomenon of coagulation is of enormous physiological importance as it prevents further haemorrhage. When bleeding occurs, the shed blood coagulates and the bleeding vessel is plugged off by the clot. The retraction of clot, compresses the ruptured vessel further and bleeding stops.

**Mechanism:**

Coagulation of blood is a complicated process in which about 13 coagulation factors are involved. All these factors are blood proteins or their derivatives. Even if one of the factor is defective, the whole clotting process is impaired leading to haemorrhage. These factors are from F-I to F-XIII.

Clotting mechanism begins by Trauma to tissues or trauma to blood. In each case it leads to formation of prothrombin activator which causes conversion of prothrombin into thrombin. There are two pathways of formation of prothrombin activator.



**i) Extrinsic Pathway**

It begins with trauma to Vascular wall or to the tissues outside the blood vessel.

**ii) Intrinsic pathway**

It begins with trauma to blood itself

In both pathways, different blood clotting factors play important roles.

Davie and Ratnoff (1965) have proposed a waterfall sequence hypothesis to explain the events taking place in coagulation process. Whereas Macfarlane has suggested a scheme of coagulation called enzyme cascade which is similar to waterfall sequence.

Blood clotting factors exist in inactive form and are activated sequentially until finally **prothrombin activator** is formed.

**Extrinsic Mechanism** ( Factors involved – III-VII-X-V) for formation of prothrombin activator

- 1) It begins with trauma to blood vessel or tissues outside the blood vessel. It releases **tissue factor and Tissue phospholipids** and clotting process starts.
- 2) The tissue factor complexes with blood clotting factor VII and activates it.

- 3) Activated factor VII in presence of  $Ca^{++}$  and tissue phospholipids acts on factor –X and activates it.
- 4) Activated factor X acts on Factor V and activates it.
- 5) Activated F-X complexes with tissue phospholipids, Factor-V,  $Ca^{++}$   
And forms a complex called prothrombin activator.
- 6) Prothrombin activator converts prothrombin in to thrombin under influence of  $Ca^{++}$
- 7) Thrombin acts on fibrinogen and converts it in to fibrin monomers
- 8) Fibrin monomers polymerize with other fibrin monomers and form long fibrin threads that form reticulum of the clot.
- 9) At first clot is weak but later on with the help of active fibrin stabilizing factor ( F- X III ) clot becomes strong.
- 10)WBCs and RBCs get trapped in to reticulum of the clot
- 11)Clots adhere to the damaged surface of the blood vessel and thereby prevents the blood loss.
- 12) Clot retraction Following clot formation, the volume of the clot decreases , this is called as clot retraction platelets are necessary for clot retraction.contain contractile protein Thrombosthenin, which contracts and reduces the volume of the clot. Following this a clear fluid is separated out called as serum.

## Extrinsic Pathway of Blood Coagulation

Truama to blood vessel/ Tissue rupture



Tissue Factor                      and                      Tissue Phospolipids

Tissue Factor(F-III) + Factor VII (Proconvertin ) → Activated Factor VII  
(Proconvertin/Stable Factor)

↙  $ca^{++}$

Acts on Factor X( Stuart Factor) → Activated Factor X

↙  $ca^{++}$

Acts on Factor V(Proaccelerin/labile Factor) → Activated Factor V

↙  $ca^{++}$

Activated Factor X+ Activated Factor V +  $ca^{++}$  + Tissue Phospolipids



Form a complex Prothrombin Activator

↓  $ca^{++}$

Prothrombin (F-II) → Thrombin

↙  $ca^{++}$

Fibrinogen( F-I ) → Fibrin monomers

↙  $ca^{++}$

Fibrin monomers → Polymerization

↓ Fibrin stabilizing factor- F-XIII

Reticulum + RBC& WBC →clot

**Intrinsic Mechanism** begins with injury to blood itself and continues through following steps ( F-III, F-XII-F-XI-F-IX-FVIII-F-X-F-V)

- 1) Trauma to blood alters two important clotting factors in the blood  
Factor XII and Platelet Phospholipids i.e. F- III
- 2)When F-XII comes in contact with collagen outside the blood vessel,it gets activated and acts as an enzyme for activation of F-XI
- 3) Damaged platelets adhere to the wet surface of blood vessel and release platelet phospholipids i.e.F- III.
- 4)Activated factor XII acts enzymatically on F-XI i.e. Plasma Thromboplastin Antecedent (PTA –Factor) and activates it.
- 5)Activated factor XI acts enzymatically on F- IX i.e. Christmas factor and activates it ( $Ca^{++}$  are necessary)
- 6) Factor IX activates F-VIII (Anti Haemophilic Factor)
- 7) activated F- IX , F-VIII and platelet phospholipids,activate factor-X .
- 8) Activated Factor X acts enzymatically on Factor V ( Proaccelarin) and activates it, ( $Ca^{++}$  are necessary).
- 9) **Activated F-V, activated X ,Platelet phospholipids and  $Ca^{++}$  form** a complex called prothrombin activator Prothrombin activator converts prothrombin in to thrombin under influence of  $Ca^{++}$
- 10)Thrombin acts on fibrinogen and converts it in to fibrin monomers
- 11) Fibrin monomers polymerize with other fibrin monomers and form long fibrin threads that form reticulum of the clot.

12) At first clot is weak but later on with the help of active fibrin stabilizing factor ( F- XIII ) clot becomes strong.

13) WBCs and RBCs get trapped in to reticulum of the clot

14) Clots adhere to the damaged surface of the blood vessel and thereby prevents the blood loss.

### **Intrinsic pathway of clotting mechanism**

Injury to blood or trauma to blood

Blood comes in contact with collagen outside the blood vessel, F-XII

get activated and damaged platelets release F-III i.e. platelet phospholipids

F-XII Acts on Factor XI (Plasma Thromboplastin Antecedent) → Activated Factor XI

↙  $Ca^{++}$

F-XI Acts on Factor IX ( Christmas Factor) → Activated Factor IX

↙  $Ca^{++}$

F-IX Acts on Factor VIII ( Anti haemophilic Factor) → Activated Factor VIII

↙  $Ca^{++}$

Activated Factor VIII + Platelet Phospholipids +  $Ca^{++}$  → Act on Factor X (Stuart Factor)

↙  $Ca^{++}$

Activated Factor X acts on Factor V → Activated Factor V (Proaccelerin)

**Activated Factor X + Factor V + Platelet Phospholipids +  $Ca^{++}$**

Form a complex **Prothrombin Activator**

↓  $Ca^{++}$

Prothrombin (F-II) → Thrombin

↙  $Ca^{++}$

Fibrinogen( F-I ) → Fibrin monomers

↙  $Ca^{++}$

Fibrin monomers → Polymerization

↓ Fibrin stabilizing factor- F-XIII

Reticulum + RBC& WBC → clot

## **Failure of clotting Mechanisms**

Blood fails to clot due to following defects:-

### **1) Lack of Fibrinogen i.e. Factor –I**

Blood fails to clot due to absence of protein Fibrinogen. In a rare congenital disease fibrinogen is lacking in the blood causing fibrinogenopenia. Such condition also occurs in abnormal pregnancy.

### **2) Diminution of Prothrombin or Factor-II**

Prothrombin is produced in liver so in liver diseases like cirrhosis of liver, cancer of liver etc. there is diminution of synthesis of prothrombin in the liver. Vitamin K helps in formation of prothrombin in liver. And it is absorbed from small intestine in presence of bile salts. In liver diseases like obstructive jaundice, liver cirrhosis etc. secretion of bile salts is affected and in absence of bile salts Vitamin K is not absorbed. Due to this synthesis of prothrombin and proaccelarin i.e. Factor V is decreased and clotting is affected.

**3) Due to lack of antihæmophilic Factor i.e. Factor VIII ( Haemophilia A)** in blood, person suffers from disease haemophilia in which blood fails to clot. Haemophilia is a hereditary disease which occurs in males but is transmitted through females. In this disease clotting time is abnormally prolonged. Patient shows tendency to bleed. Haemophilic person dies very early life due to repeated hemorrhage.

**4) Due to diminution of Factor V, VII, IX (Haemophilia B) XI (Haemophilia C)** in blood person shows pseudohaemophilia and develops a tendency to bleed.

**5) Thrombocytopenia** means presence of very low quantities of platelets in the circulatory system. Blood fails to coagulate. Bleeding occurs from small capillaries therefore there is hæmorrhage all over the body. Person displays thrombocytic purpura.

Platelets are important in repairing minute capillaries. They aggregate and plug small bleeding vessels. Normal count of platelets 200000 to 400000 /cumm.when count falls below 50000 /cumm.,blood coagulation is affected.

#### **6)Vitamin K deficiency**

Vit.K is necessary for some intermediate stages in formation of clotting factor.It is continuously synthesized in the gastrointestinal tract by bacteria and absorbed in blood along with fat. Vit K deficiency occurs due to poor absorption of fat by gastrointestinal tract.In liver diseases bile production is decreased hence fat digestion is affected which in turn affects absorption of vit K.

**Anticoagulants:** Substances which prevent coagulation of blood are called as anticoagulants.

Richard Lewshan discovered anticoagulants in 1914. discovered that ,Blood remains in fluid state when mixed with citrate solution. This led to opening of blood banks during 1<sup>st</sup> world war.

There are four types of substances which prevent coagulation of blood . These are:

1. Natural Anticoagulants
2. Anticoagulants used in blood banks a) A.C.D. b) C.P.D.
3. Anticoagulants used in laboratory
4. Therapeutic anticoagulants

**Natural Anticoagulants** : these are present within the body hence the name natural anticoagulants. They keep the blood in fluid state in the vessels.

Physical characteristic of endothelium of blood vessel

**Smoothness of the vascular Endothelium** prevents contact activation of the intrinsic clotting mechanism. As soon as endothelium of blood vessel is damaged, the clotting mechanism is initiated.

**Monomolecular layer of negatively charged proteins** absorbed on the inner surface of the endothelium repels the clotting factors and blood platelets and prevent clotting. As soon as the endothelium of the blood vessel is damaged , its smoothness and negative charge are lost and intrinsic pathway is activated.

**Antithrombin III** : This is normally present in the blood. Antithrombin III inhibits the activation of IX, X, XI, XII factors. Antithrombin III action is facilitated by heparin. Its deficiency leads to venous thrombosis.

**Heparin** : To maintain blood in a fluid state in blood vessels, blood contains an anticoagulant called as heparin. It is a powerful anticoagulant but has short duration of activity. It is secreted by the basophil or mast cells. These cells contain granules which are supposed to be the precursor of heparin. Heparin helps to maintain the normal fluidity of the blood. It inhibits transformation of prothrombin into thrombin.

**Protein 'C'** : Thrombin in combination with thrombomodulin (protein present in plasma) activates protein C which inhibits activated factor VIII and V.

**$\alpha$ -2 macroglobulin** : is a large globulin molecule. This has also anticoagulant property. It acts as a binding agent for coagulation factors until they are destroyed.

**Fibrin threads** : 85 to 90% thrombin formed from prothrombin is absorbed on fibrin threads and thus they prevent spreading thrombin into remaining blood and prevent excessive spread of blood clot.

**Antithrombin heparin co-factor**: also acts as an anticoagulant. Thrombin which does not get absorbed on fibrin threads combines with co-factor and gets inactivated.

**Fibrinolytic System** : exists in the body which brings about clot lysis. Small clots are immediately lysed by this system. Plasminogen co-precipitates with fibrin as plasmin. When activated, the plasmin in clots digests the fibrin into soluble fragments, dissolving the clots.

### **Anticoagulants used in Blood banks**

- a) Acid citrate Dextrose (ACD) : It forms a complex with  $Ca^{++}$  and decreases its level.

- b) Citrate Phosphate Dextrose (CPD) : Mechanism is similar to ACD CPD is better than ACD as the O<sub>2</sub> transport function is better preserved by CPD.
- c) CPD is added to collected blood to store the blood up to 14 days. It binds with plasma ca ions

### **Anticoagulants used in Laboratory:**

In laboratory Citrate or oxalate of Na ( 3.8%)or potassium (3%), 0.3% Sodium Fluride , EDTA are used as **Anticoagulants for various investigations like ESR, Blood urea etc.**

By adding various salt solutions like 1/4<sup>th</sup> of the blood volume of magnesium sulfate or equal volume of half saturated Sodium sulfate soln. , clotting is prevented.

### **Therapeutic anticoagulants**

These substances are used to prevent thrombus formation in vivo.

- a) Heparin : given intravenously
- b) Dicoumarol :It is a synthetic product. Patients with hyper coaguly are given this anticoagulant for preventing the formation of thrombus.. It prevents synthesis of clotting factors mainly prothrombin as it is antagonistic to Vit -K
- c) Thrombin is sprayed on the bleeding surfaces along with fibrinogen to arrest the bleeding.
- d) Foam of fibrin can be spread on the bleeding surface.
- e) Sodium alginate when comes in contact with blood it is convered in to calcium alginate which clots and forms tenacious layer thus prevent bleeding.
- f) Cellulose gauze made upof oxydised cellulose swells when socked with blood and prevents further bleeding.

**Some other substances of biological origin which also serve as anticoagulants are,**

Protamine a simple protein found in fish

Peptones when injected into veins

Extract of cray fish and mussels ( Sea clam) increase the secretion of heparin by mast cells.

Hirudin , leech extract

Venom of certain snakes inhibit activation of prothrombin and thrombin fibrinogen reaction.

Azodyes, synthetic products like Chicago blue, trypan red, trypan blue act as anticoagulants.