DEFINITION, COMPONENT, PLATELETS & BLOOD VESSELS

COAGULATION AND FIBRINOLYSIS

HAEMOSTASIS I & II BY DR. KIBET SHIKUKU

LECTURE OBJECTIVES

- Define haemostasis
- Name components of haemostasis
- Describe the role of the platelets and blood vessels

DEFINITION

- Maintenance of blood in the fluid state, flowing and prevention of blood loss from the blood vessels (natural physiological state)
- A dynamic process which involves a balance between pro-cagulant and anti-coagulant mechanisms

INTRODUCTION

- Imbalance of haemostasis:
  - Prolonged bleeding – haemorrhage
  - Undesired clotting in the blood vessels – thrombosis
  - Without this balance the individual may experience bleeding (poor clot formation or excessive fibrinolysis)
  - Vaso-occlusion (uncontrolled formation of thrombin in the vascular system, occluding vessels and depriving organs of blood)
- Other functions of the haemostatic mechanism:
  - Part of the immune system
  - Tissue healing and repair

COMPONENTS OF HAEMOSTASIS

1. Blood vessel endothelium – besides lining the vessel, it is also a storage for coagulation factors
2. The platelets
3. Coagulation system
4. Natural anticoagulants
5. Fibrinolytic system

PLATELETS

- Numbers:
  - CBC /FBC/ Full blood hemogram
    - Platelet count
      - Too many – thrombosis
      - Too little - haemorrhage
    - Platelet morphology
      - Anucleated
- Function
  - Bleeding time (BT) – prick skin and check the time it takes for blood to clot; assesses the platelet as well as the blood vessel.
  - Platelet aggregation
    - Whole blood aggregation
    - Platelet-rich plasma aggregation

PLATELETS CONTRIBUTE TO HAEMOSTASIS IN 2 MAIN WAYS:

- Primary haemostatic plug:
  - Adhesion
  - Aggregation
  - Secretion
- Secondary haemostatic plug:
  - Pro-coagulant activities are generated

PLATELET PLUG FORMATION: ADHESION

- Platelets bind to exposed adhesive sub-endothelial connective tissue:
  - Collagen
  - Von Willebrand's factor (vWF)
  - Fibronectin
- Mechanisms components:
  - vWF: Links platelets to endothelial binding site
  - Platelet receptor **GP1b (Glycoprotein Ib)**
  - Collagen fibres - GPIa facilitates platelet adhesion to collagen
- Actin contracts and pseudopds form
- Initial activation of platelets is reversible but over time the changes become irreversible:
  - Facilitates activation

AGGREGATION

- Platelet-platelet interaction
- Mechanism components:
  - ATP
  - Ionized calcium
  - Fibrinogen
  - Platelet receptor **GPIIb/IIIa**
- Initial aggregation –reversible
- Secondary aggregation – irreversible (the transformation of irreversible aggregated platelets into a mass of degenerative platelet material without membranes is termed **viscous metamorphosis**; by platelet lysosomes) – white clot is formed which is the primary haemostatic plug.

COAGULATION SYSTEM

- Composed of plasma proteins called **coagulation factors** found in the inactive form in small amounts in plasma which on activation have a cascading effect (activate a substrate into active form in turn activates the next substrate)
- Majority are glycoproteins in nature synthesized in the liver, vessel endothelium, some ~~ate~~ elaborated by platelets
- Activation does not occur in solution but on surfaces.

- <u>Coagulation factors</u>
  - Numbered as FI to FXIII; FI, II & III are found in circulation
    - FI – fibrinogen
    - FII – Prothrombin
    - FIII – Tissue factor
    - FV – Proaccelerin
    - FVII – Proconvertin
    - FVIII – Anti-haemophilic factor
    - FIX – Christmas factor
    - FX – Stuart factor
    - FXI – PTA
      FXII - Hageman factor

CONT.

- Prekallikrein (Fletcher factor)
- HMW kininogen
- Cofactors:
  - Calcium ions
  - Phospholipids
- Contact factors – **FXII, XI, Pre-kallikrein, HMW Kininogen**
- Vitamin K dependent factors – **FII, VII, IX, X;** synthesized in the liver (are serine proteases) bind to Ca++ and phospholipids for activation, require vitamin K for gamma carboxylation of terminal amino acids residues
- Thrombin sensitive factors – activated by thrombin **FI, FV, FVII, FXIII and Tissue factor**
- Fibrinogen – **FI** (Plasma protein -> 2-4g/L)

THE COAGULATION CASCADE

- Activation of the coagulation factors
- Propagation – other factors are activated
- Amplification
- Fibrin formation
- Modulation (inhibition) by natural inhibitors present in plasma

- On the basis of activation process; divided into:
  - Extrinsic pathway
  - Intrinsic pathway
  - Common pathway

EXTRINSIC PATHWAY

1. After tissue injury, **Tissue factor (FIII)** is released
2. It converts **FVII** to **FVIIa** which is active
3. **FVIIa** acts on **FX** converting it to **FXa**, a process that requires **Ca++ and phospholipids** (from the platelets)

Common pathway:

1. **FXa** activates **FII** (Prothrombin, FII, to thrombin, FIIa) in the presence of **FVa, phospholipids and Ca++**
2. **FIIa** acts on **fibrinogen, FI** converting it to **fibrin monomers**
3. **FIIa** also activates **FXIII** in the presence of **Ca++** and **FXIIIa** polymerizes fibrin monomers to form a **stable fibrin clot.**

INTRINSIC PATHWAY

- Requires contact
- **Pre-kallikrein** is activated to **kallikrein**
- **Kallikrein** activates **FXII**
- **FXIIa** converts **FXI** to **FXIa**
- **FXIA** converts **FIX** to **FIXa**
- **FIXa** converts **FX** to **FXa** also requiring **FVIII, calcium, phospholipids.**

- **NOTE**
  - The common pathway begins from FX
  - In deficiency of FVII, the intrinsic pathway is affected

THE FIBRINOLYTIC SYSTEM

- Process by which fibrin is broken down to soluble degraded products (FDPs, D dimers/ cross-linked fibrin)
- Ensures patency of blood vessels (excessive lysis in pathological states leads to bleeding)
- Role by plasmin – a serine protease; exists in plasma in inactive form as plasminogen
- **Tissue Plasminogen Activator** (tPA) converts **plasminogen** to **plasmin** which acts on the fibrin clot breaking it down into small components; **streptokinase** and **urokinase** are activators of tPA (tPA – like)

INHIBITORS OF THE COAGULATION FACTORS

- Tissue factor pathway inhibitor – inhibits the extrinsic pathway; natural within our systems and is activated as the tissue factor gets activates
- Inhibitors of the intrinsic pathway:
  - **Protein S** carrying **protein C** (released after cleaving)
    - Protein C acts at the level of **FIX**
    - Both also inhibit FVa and FVIIIa
- Anti-thrombin III – inhibits the common pathway (thrombin inhibitor)' inhibits FIIa, FXa
- Other natural anticoagulants: **Heparin cofactor II** (both commercial and natural), **Protein Z**
  - Heparin potentiates antithrombin
- Inhibitors of the fibrinolytic system – **alpha-1 macroglobulin, alpha-1 antiplasmin, alpha-1 antitrypsin, C1 inactivator** (inhibits plasminogen activators)

LABORATORY TESTS OF HAEMOSTASIS

- Screening tests (Coagulation screen)
  - FBC & PBF
  - BT – assesses blood vessel function and platelet numbers and function; standard template, Ivy, Quick`s, Duke`s method)
  - Prothrombin Time (PT) – assesses the **extrinsic pathway**
    - Prolonged PT – FVII affected
  - APTT/KCCT – Activated Partial Thromboplastin Time/Kaolin Chephalin Clotting Time – assesses the **intrinsic pathway**
    - Prolonged APTT/KCCT – FXII, Xi, IX, VIII affected
  - Thrombin time (TT) – assesses the **common pathway**
    - Prolonged TT – Fibrinogen affected
- **NOTE: If all tests are abnormal, most likely FI affected**

CONT.

- Specific tests:
  - Platelet function tests: adhesion, aggregation
  - Tests for coagulation
    - Substitution tests – based on APTT
    - Factor assays
  - Tests for fibrinolytic activity
    - Whole blood clot lysis
  - Euglobin clot lysis time
  - Plasminogen assays
  - Fibrinogen Degradation Products (FDP) Assays
  - D-dimer assays
  - Plasminogen assays
  - Assays for physiological inhibitors of coagulation – antithrombin III, Protein C & S.

QUESTION

- I have a patient with prolonged **APTT** mixed with **FVIII** -deficient plasma ending up with a normal **APTT**
- Take the same abnormal plasma and mix with **FIX** deficient plasma and it does not correct
- Missing factor?
  - **FIX**
  - Using FVII deficient plasma avails the missing factor
  - Bringing in plasma FIX deficient plasma, when our patient is missing FIX does not improve the defect hence the APTT is still prolonged.

HAEMATOLOGY PRACTICAL SESSION

MEGAKARYOCYTE

- Abundant cytoplasm
- Multiple nuclei which bud off to form the platelet

- ASSIGNMENT:
- Find out conditions that cause the following in the BM:
  - Abnormal megakaryocytes
  - Increased megakaryocytes
  - Reduced megakaryocytes
- Read about **ITP (Idiopathic thrombocytopenic purpura)**

ASSIGNMENT 1

- A 5 year old male with recurrent swelling of the knee. A deceased maternal uncle had a similar problem:
  - BT - 6 minutes
  - PT - 14 seconds (control 15 s)
  - KCCT - 95 seconds (control 40s)
  - TT - 11 seconds (control 10s)
  - Blood film - Normal appearance
- Name 2 conditions that may give rise to this abnormality

ASSIGNMENT 2

- An adult male ad unscreened blood transfusion as an emergency following a RTA 6 months ago. Now the patient is noted to have yellow eyes and is having a bleeding tendency
  - Hemogram – Normal
  - PBF – Normal
  - BT – Normal
  - PT – 29s (control – 12s)
  - KCCT/APTT – 96 s (control – 43s)
  - TT – 17 s (control – 10s)
- A) Indicate any abnormal laboratory findings
- Outline the patho-physiology of these abnormalities
- What further tests would you carry out in this patient.

ASSIGNMENT 3

- A 12 year old female with recurrent epistaxis all her life
  - TBC (including platelet count) – Normal
  - BT (Ivy's method) – 19 minute
  - PT – 13 seconds (Control – 13.5s)
  - KCCT – 81 seconds (Control 41 s)
  - TT – 11 seconds (control 10s)
- Common on the above results
- What is the likely diagnosis
- What is the mode of inheritance of the disorder

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LABORATORY ASPECTS OF HAEMOSTASIS

LEARNING OBJECTIVES

- Describe the role of the laboratory in the patient management
- Describe pitfalls in haemostasis testing
- Describe the first line tests in bleeding disorders
- Describe specific tests that are carried in patients with bleeding disorders
- Describe the tests carried out for anticoagulant monitoring at KNH

ROLE IN THE LAB IN PATIENT MANAGEMENT

- Diagnosis of haemostasis disorders
- Preparation of patients for operations including biopsies, physiotherapy
- Monitoring of patients with bleeding disorders during operations
- Investigating for thrombophilias (genetic & acquired)
- Monitoring of patients on anticoagulant therapy.

CAUSES IF ERRORS IN LAB RESULTS OF HAEMOSTASIS

- Blood collection
- Anti-coagulant – sodium citrate solution
- Delays in delivering specimen to the lab
- Reagents
- Equipments
- Competence of the lab personnel
- Post-analytical – clerical errors

PRE-ANALYTICAL ERRORS

- Specimen collection errors
- Wrong specimen containers

BLEEDING DISORDERS

- Examples include:
  - Von Willebrand's Disease
  - Immune thrombocytopenia (mucosal hemorrhages)

LAB & BLEEDING DISORDERS

- Screening tests
  - FBC & Platelet count
  - Bleeding time
  - Prothrombin time
  - APTT (KCTT)
  - Thrombin time
- Specific tests
  - Factor assays
  - vWF (von Willebrand's Factor)
  - D dimers
  - Platelet aggregation assays

THROMBOSIS

- Monitoring for anticoagulants
  - AOTT (KCCT)
  - INR (International Normalized Ratio)
- Thrombophilias – Protein C & S, ATIII
  - Anti-phospholipid antibodies

SUMMARY

- Causes of errors in haemostasis testing
- Approach in laboratory testing of a patients
- Screening (first line) haemostasis tests in bleeding disorders
- Specific tests in patients with bleeding disorders
- Tests used in monitoring anti-coagulant therapy

BY DR. GITHANGA

ABNORMAL COAGULATION

LECTURE OBJECTIVES

- Classify bleeding disorders
- Pathogenesis of the bleeding disorders
- Clinical and laboratory features of the bleeding disorders
- Thrombotic disorders

CLASSIFICATION

- Vessel wall disorders
- Platelet disorder
- Coagulation factor disorders
- Fibrinolytic disorders
- These may be inherited or acquired

VASCULAR DISORERS

- May be acquired or inherited
- Bleeding is usually mild and confined to the skin, mucosa & gingiva

- **Inherited vascular disorders**
  - Hereditary haemorrhagic telengiectasis (Rendu-Osler-Weber syndrome)
  - Giant Cavernous Hemangioma (Kasabach-Merrit syndrome)
  - Connective tissue disorders: Ehlers –Danlos syndrome and Pseudoxanthoma elasticum

ACQUIRED VESSEL WALL ABNORMALITIES

- Senile purpura
- Vasculitis associated with infections
- Anaphylactoid purpura
- Vitamin C deficiency
- Cushing's syndrome – causes thinning of the skin
- Drug associated e.g. steroid therapy

CLASSIFICATON OF PLATELET DISORDERS

- Hereditary
  - Quantitative
    - Thrombocytopenia
    - Thrombocytosis
  - Qualitative
- Acquired
  - Quantitative
    - Thrombocytopenia
    - Thrombocytosis
  - Qualitative
- NOTE: **Acquired disorders are more common; disorders may be both qualitative & quantitative esp. the hereditary disorders.**

QUALITATIVE PLATELET DISORDERS

- May result from defects in any of the 3 critical platelet reactions:
  - Adhesion
  - Aggregation
  - Granule release
- Platelet disorders may occur alone or together with coagulation defects.

CONGENITAL PLATELET DISORDERS

- Glanzmann's thrombasthenia (defective aggregation)
- Bernard-Soulier syndrome (defective adhesion)
- Storage pool syndrome (absence of dense granule contents)

ACQUIRED DISORDERS (MORE COMMON): THROMBOCYTOPENIA

- Defective platelet production e.g. BM failure
- Increased destruction e.g. immune destruction e.g. idiopathic (immune) thrombocytopenic purpura
- Increased sequestration in the spleen

FUNCTIONAL DEFECTS

- Drug-induced (e.g. by Aspirin, NSAIDs)
- Renal disease – uraemia inhibits platelet function
- Liver disease
- Myeloma – plasma cell proliferative disorders
- Myeloproliferative disorders e.g. essential thrombocythemia

CONGENITAL COAGULOPATHIES

- Coagulation defects: hereditary
  - Haemophilia A, B
  - Von Willebrand's disease
  - Other rare factor deficiencies FVII, fibrinogen, FV etc.

HAEMOPHILIA

- Hereditary bleeding disorder due to the deficiency of a blood clotting factor
- Commonest severe hereditary bleeding disorder
- Inherited in a **sex-linked recessive** fashion (males affected more compared to females)

TYPES OF HAEMOPHILIA

- Classical haemophilia (Haemophilia A)
  - Caused by factor VII deficiency
  - 8-10 times more common
- Christmas disease (Haemophilia B)
  - Due to factor IX deficiency

VON WILLEBRANDS'S DISEASE

- Deficiency of vWF
- Inherited as an autosomal dominant
- Most common inherited disease, usually not severe
- Presents with increased and prolonged bleeding after trauma/surgery, easy bruising etc.

ACQUIRED COAGULATION DEFECTS

Liver disease

- Most coagulation factors are produced solely in the liver
- Severe hepatic disease associated with:
  - Impaired production of coagulation & fibrinolytic system factors
  - Vitamin K deficiency

Renal disease

- Bleeding tendency associated with acute and chronic renal disease

Other conditions:

- Massive transfusion (dilution of coagulation factor)
- Haemorrhagic disease of the newborn (Vitamin K deficiency)

DIC

- This is a disorder characterized by inappropriate diffuse fibrin deposition in the microvasculature leading to consumption of the coagulation factors
- Concurrent fibrinolysis.
- Causes:
  - Septic abortions
  - Malignancies,
  - Toxins
  - Severe liver disease
  - Hemolytic transfusion reaction
  - Tissue injury

CLINICAL MANIFESTATIONS

- Clinical manifestations of bleeding disorders can involve various systems
- Depends on the extend and type of disease
- Mild disease may present with no clinical signs
- Severe coagulopathies may have different features
- When skin and mucosa are involved
  - Petechiae, ecchmoses, angioma, hematomas.
- Deep hematomas & hemarthroses of major joints seen in severe hemophiliacs
- Disorders of platelet quantity may result in mucocutaneous bleeds e.g. epistaxis, gingival bleeding & risk of haemorrhage & stroke.
- Conditions include: Arthropathy, haemarthrosis

LABORATORY TESTS: DIAGNOSIS

- COAGULATION SCREEN
  - FBC with platelet count
  - Bleeding time (BT): tests for platelet function & number and vascular integrity
  - Prothrombin time (PT): tests for factor VII
  - APTT (Activated Partial Thromboplastin Time): tests for intrinsic pathway picking up factors VII & IX
  - Thrombin time (TT): sensitive to fibrinogen abnormalities

OTHER TESTS

- Mixing tests
- Fibrinogen degradation products: D-dimers
  - Important in DIC
- Factor assays
  - FVIII, FIX
- Platelet function tests

THROMBOTIC DISORDERS

- Hypercoagulability – a state of heightened activation of the coagulation system
- May be due to inherited or acquired factors
- These inherited (thrombophilias) and acquired conditions most commonly present as **venous thromboembolism.**

THROMBOTIC DISORDERS

- Inherited
  - Protein C deficiency
  - Protein S deficiency
  - Antithrombin deficiency
  - Abnormal factor V (FV Leiden)
- Acquired
  - Vascular disorders
  - Stasis
  - Hyper-viscosity
  - Platelet abnormality
  - Cancer
  - Others

THE END ☺