CLINICOPATHOLOGICAL PROFILE OF PATIENTS DIAGNOSED WITH CONGENITAL DISORDER OF HAEMOSTASIS

by

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A thesis submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY, CHENNAI

in partial fulfillment of the requirements for the award of the degree of

M.D in PATHOLOGY

DEPARTMENT OF PATHOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

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CERTIFICATE I

This is to certify that the dissertation work entitled **"Clinicopathological Profile of Patients Diagnosed with Congenital Disorder of Haemostasis"** submitted by **Dr. G. Saranyadevi,** is a bonafide work done by her, during the post-graduation study period in the department of Pathology of PSGIMS&R, from 2017 to 2020. This work was done under the guidance of **Dr. T. M. SubbaRao,** Professor & Head, Department of Pathology, PSGIMS&R.

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CERTIFICATE II

This is to certify that this dissertation work titled **"Clinicopathological Profile of Patients Diagnosed with Congenital Disorder of Haemostasis"** of the candidate **Dr. G. Saranyadevi,** with registration Number **201713403** for the award of MD degree in the branch of Pathology. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **Three (3)** percentage of plagiarism in the dissertation.

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DECLARATION

I, **Dr. G. Saranyadevi,** do hereby declare that the thesis entitled **"Clinicopathological Profile of Patients Diagnosed with Congenital Disorder of Haemostasis"** is a bonafide work done by me under the guidance of Dr T M SubbaRao, Professor & Head, in the Department of Pathology, PSG Institute of Medical Sciences &Research. This study was performed at the PSG Institute of Medical Sciences &Research, Coimbatore, under the aegis of the The Tamilnadu Dr MGR Medical University, Chennai, as part of the requirement for the award of the MD degree in Pathology.

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To
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Ref: Project No. **17/387**

Date: December 29, 2017

Dear Dr Saranyadevi,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 08.12.2017 to conduct the research study entitled "*Clinicopathologic profile of patients diagnosed with congenital disorder of hemostasis*" during the IHEC meeting held on 22.12.2017.

.The following documents were reviewed and approved:

1. Project submission form
2. Study protocol (Version 1 dated 08.12.2017)
3. Application for waiver of consent
4. Confidentiality statement
5. Data collection tool (Version 1 dated 08.12.2017)
6. Permission letter from the Dean
7. Current CVs of Principal investigator, Co-investigator
8. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 22.12.2017 at IHEC Secretariat, PSG IMS & R between 10.00 am and 11.00 am:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr R Nandakumar (Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
2	Dr D Vijaya (Member – Secretary, IHEC)	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes
3	Dr S Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr Sudha Ramalingam	MD	Epidemiologist, Ethicist Alt. member-Secretary	Female	Yes	Yes
5	Dr G Subhashini	MD	Epidemiologist	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date

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1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
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   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
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   f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review
7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Thanking You,

Yours Sincerely,

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Firstly, I would like to thank Almighty God, without whose generous blessings and caring hand I would not have reached my present position.

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Last but not the least, my gratitude is to all those **patients** who have believed in our diagnostic services, provided all the clinical details and underwent patiently all the tests needed for a final diagnosis. I pray for their health and an uneventful journey in life.

ADP	Adenosine diphosphate
AD	Autosomal Dominant
AIIMS	All India Institute of Medical Sciences, Delhi
AR	Autosomal Recessive
ATP	Adenosine triphosphate
APCC	Activated Prothrombin complex concentrates
CDH	Congenital Disorders of Hemostasis
CMC	Christian Medical College, Vellore
DDAVP	Desmopressin
EDTA	Ethylene DiamineTetraacetic Acid
ELISA	Enzyme Linked Immuno Sorbent Assay
FFP	Fresh Frozen Plasma
GP	Glycoprotein
IBD	Inherited Bleeding Disorder
INR	International Normalized Ratio
PCC	Prothrombin Complex Concentrates
PPP	Platelet Poor Plasma
PT	Prothrombin time
APTT	Activated partial thromboplastin time
SPD	Storage Pool Disorders
rFVIIa	Recombinant Factor VIIa
vWF	von Willebrand Factor
vWD	von Willebrand disease
XLR	X-Linked Recessive

Bleeding happens secondary to trauma or surgery. It is generally controllable with standard hemostatic measures. However, when the quantity of bleeding is disproportionate to the injury it is abnormal and needs to be investigated.

The only spontaneous bleeding that is considered physiological is menstrual bleeding. Any other form of spontaneous bleeding or excess of menstrual bleeding is considered abnormal and needs to be investigated.

Both the situations mentioned above require a good clinical evaluation first. This is because the tests Prothrombin Time (PT) & Activated Partial Thromboplastin Time (APTT), considered as standard coagulation tests, are limited in their ability to reflect truly, the in - vivo hemostatic response. A bleeding history when performed carefully can differentiate between an acquired and congenital disorder of hemostasis [(1)]. In the former, it would be a waste of time and resources if these patients are subject to investigations.

The Clinical Pathology and Haematology Laboratory of our organization has in the recent past acquired a high - end coagulation analyzer with an integrated platelet function- analyzer. This has helped in the resolution of many cases that are worked up for abnormal bleeding.

Literature is replete with information on the causes for the Congenital Disorders of Hemostasis (CDH) in their respective populations. Most of the literature is from the West, where, von Willebrand Disease (vWD) is the

most common Inherited Bleeding Disorder (IBD) [2]. There are very limited publications from India on this entity. Most of these have been from AIIMS, New Delhi and CMC Vellore.

This study has attempted to observe the occurrence, causes and presentation of Inherited Bleeding Disorders in our population (Western Tamil Nadu). We have also looked into a possible association between the severity of factor deficiency and severity / type of bleeding.

1. To observe the occurrence of patients diagnosed to have congenital disorder of hemostasis among those patients being investigated for abnormal bleeding.
2. To observe the causes for the Congenital Disorder of Hemostasis.
3. To analyze the clinicopathological findings of patients diagnosed with Congenital Disorder of Hemostasis / Inherited bleeding disorders

ETYMOLOGY OF HEMOSTASIS:

The term haemostasis is a new Latin word derived from the Greek root which means halting (stasis) of blood (heme).

PHYSIOLOGY OF HEMOSTASIS:

If the blood vessel is injured or has been cut, hemostasis is achieved by many mechanisms which include the following.

1. Vascular constriction
2. Formation of platelet plug (primary haemostasis)
3. Formation of a blood clot (secondary haemostasis)
4. Fibrous tissue growth into the blood clot which leads to permanent closure of the hole in the vessel[3]

1) VASCULAR CONSTRICTION

As soon as the blood vessel is ruptured, there is contraction of the smooth muscle in the vessel wall. This contraction reduces blood flow from the injured vessel. This contraction results from

i. Local myogenic spasm
ii. Release of local autacoids from platelets and traumatised tissues
iii. Nervous reflexes

The nervous reflexes are initiated either by sensory impulses from injured vessels / adjacent tissues or by pain nerve impulses. Because of the direct damage to the vessel wall, more vasoconstriction occurs due to local myogenic spasm of the vessel. In case of smaller vessel, much more vasoconstriction is due to platelets which release Thromboxane A2, a potential vasoconstrictor.

The degree of vascular spasm is directly proportional to the severity of the vessel injury. The time period of the spasm may vary from minutes to even hours. During this time platelet plug formation and clotting takes place.

2) FORMATION OF PLATELET PLUG (PRIMARY HEMOSTASIS)

It is essential to review the physiology of the platelets to understand its role in primary haemostasis.

Physical & Chemical characteristics of Platelets:

Platelets are formed from megakaryocytes in the bone marrow. Platelets are minute discs, whose size ranges from 1 to 4 micrometers. Although platelets do not have nuclei, they have functional characteristics of cells [(4)].

The cytoplasm of platelet has the following.

i. Contractile proteins – Actin, myosin, thrombosthenin

ii. Enzymes synthesised by residuals of endoplasmic reticulum & Golgi apparatus , storage of calcium ions

iii. Mitochondria & enzyme systems synthesise Adenosine triphosphate (ATP) & Adenosine diphosphate (ADP)

iv. Prostaglandins synthesised by various enzyme systems

v. Fibrin stabilising factor

vi. Growth factor

Platelet membrane has the following.

1. *Glycoprotein coat* which prevents adhesion to normal endothelium and also causes adherence to injured endothelium.
2. *Phospholipids* that activate the clotting mechanisms

The half life of the platelet is 8 to 12 days in the circulation. After that, it is removed by tissue macrophage system. About one third of the platelets are removed by splenic macrophages [(5)].

Mechanism of Platelet Plug Formation:

After a vascular injury, platelets come in contact with the subendothelial connective tissue constituents such as collagen and von Willebrand Factor (vWF). Soon after the contact with these proteins, platelets undergo a sequence of events that results in platelet plug formation. These changes are

i. Platelet adhesion

ii. Shape change

iii. Granule release

iv. Platelet recruitment

v. Platelet aggregation

i. Platelet adhesion

Platelet adhesion is mediated by interaction of platelets with vWF which acts as a bridge between the exposed collagen and platelet surface receptor Glycoprotein 1b (Gp1b).

ii. Shape change

After adhesion, platelets change their shape from smooth discs to spiky sea urchin with increased surface area. This leads to alterations in glycoprotein IIb /IIIa which increases affinity for fibrinogen. There is translocation of negative charge to phospholipids on the surface of the platelets. The phospholipids bind to calcium and serve as nucleation sites for coagulation factor complexes.

iii. ***Granule release***

Along with shape change, platelet releases its granules. These two events (i.e. shape change and granule release) are together called Platelet

activation. Thrombin and ADP released by dense granules of platelets trigger platelet activation.

iv. ***Platelet recruitment***

Platelet activation and ADP release begets more and more platelets resulting in Platelet recruitment.

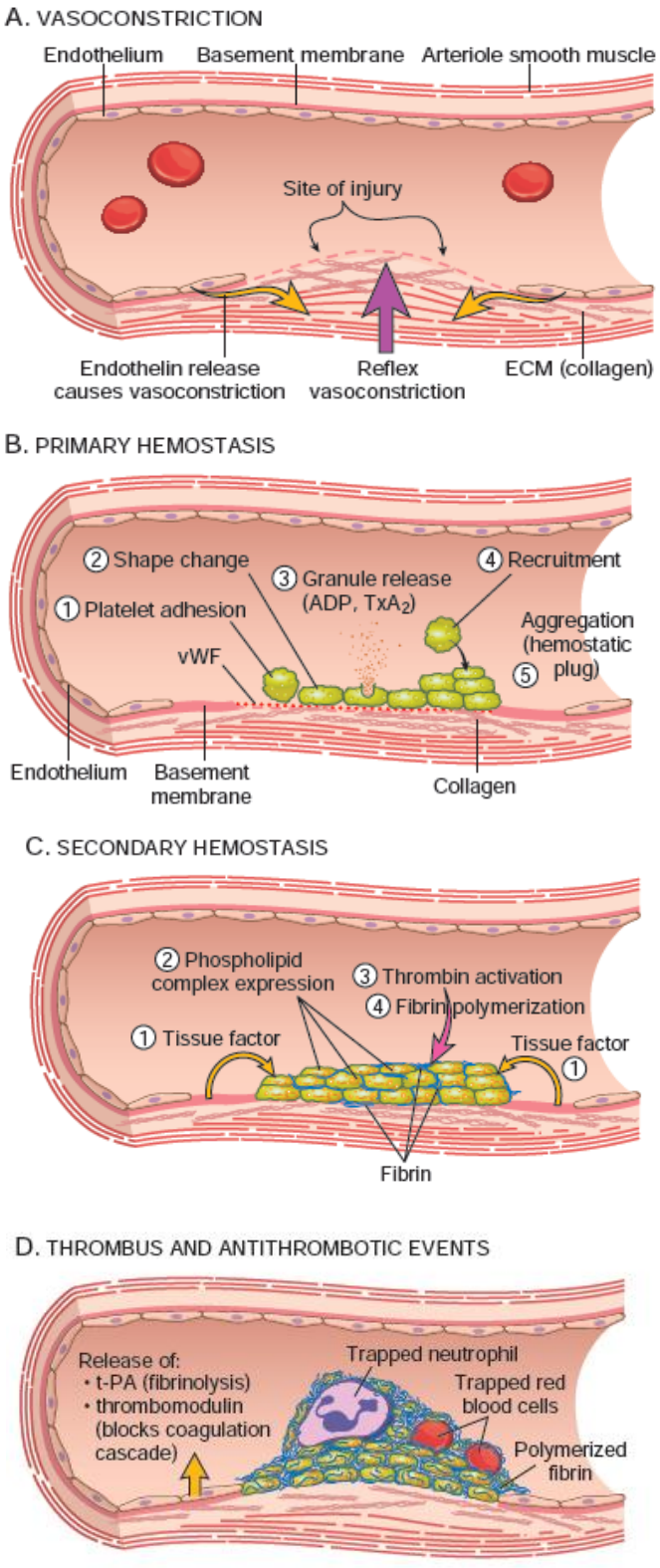
v. Platelet aggregation

Besides platelet activation there is a conformational change in glycoprotein IIb /IIIa which increases the affinity for fibrinogen, a large bivalent plasma polypeptide. This fibrinogen forms a bridge between adjacent platelets and these results in Platelet aggregation.

At the initial stages the platelet aggregation is reversible. The concurrent activation of thrombin causes platelet plug stabilization by further platelet activation and aggregation. It also promotes irreversible platelet contraction.

FORMATION OF A BLOOD CLOT (SECONDARY HAEMOSTASIS)

Thrombin converts fibrinogen to insoluble fibrin which helps in cementing the platelets in place and form a definitive secondary hemostatic plug. Hemostatic plug also contain entrapped red cells and leucocytes [(3)]



*(**Source of picture:** Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran. Pathologic basis of disease. 9th ed. Elsevier / Saunders; 2015).*

Coagulation cascade is a chain of amplifying enzymatic reaction which leads to formation of an insoluble fibrin clot. The core components of coagulation system are core proteins which causes the formation of insoluble fibrin by complex interplay of reactions.

Clotting Factors (Coagulation Proteins)

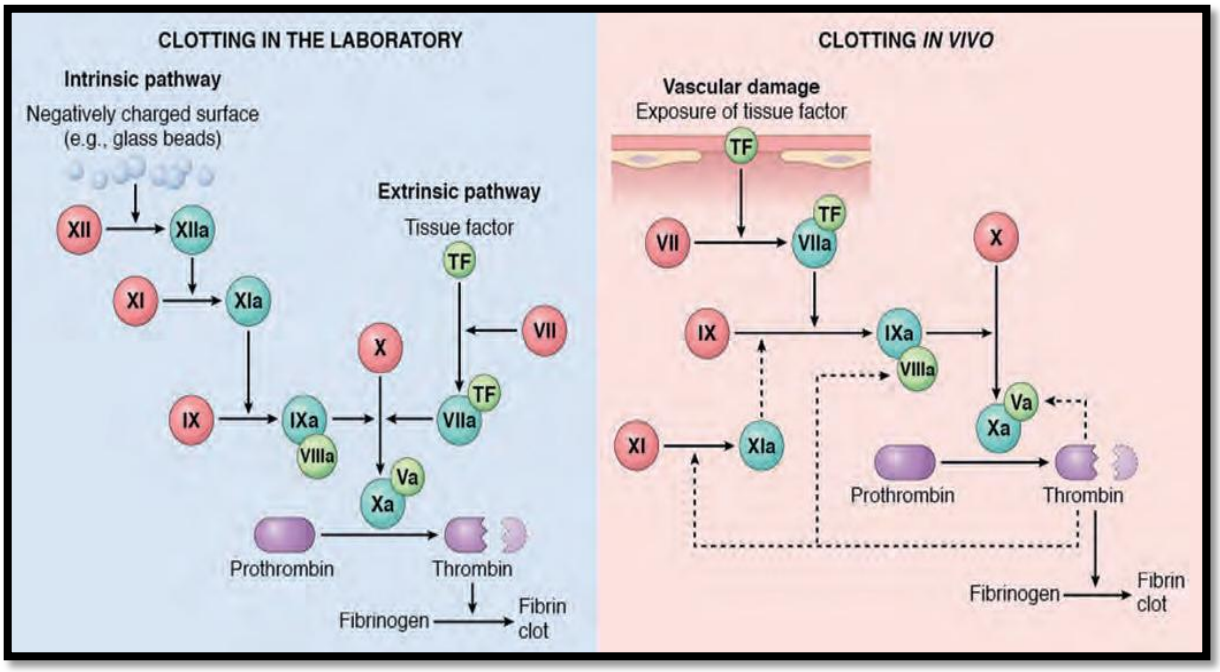
Most of the clotting factors are zymogens which are precursor forms of proteolytic enzymes circulating in inactive form.

Liver produces most pro-coagulant and anticoagulants except Factor III, IV and VIII.

In coagulation pathway each reaction step needs the following

- Enzyme (activated coagulation factor)
- Substrate
- Co factor (Reaction accelerator)

Activated platelets provide a negatively charged phospholipid surface which act as a platform for assembly of these reaction complexes. These complex proteins undergo a post transitional modification (Vitamin K dependent ϒ carboxylation of glutamic acid residues) which allows them to bind calcium and other divalent cations & participate in clotting cascade.



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Extrinsic pathway

First step in plasma mediated haemostasis. Tissue factor (TF) expressed in endothelial tissue activates extrinsic pathway**.** Normal vascular endothelium decreases the contact between TF and plasma procoagulants in normal circumstances. In case of vascular injury tissue factor (Factor III) is exposed which binds to Factor VII and calcium to promotes the conversion of factor VII to VIIa.

Intrinsic pathway

It begins with factor XII, HMW kininogen, prekallekerin and factor XI which results in activation of factor XI. Activated factor XI in turn activates

factor IX, which acts with its cofactor (factor VIII) to form tenase complex on a Phospholipid surface to activate factor X.

Common pathway

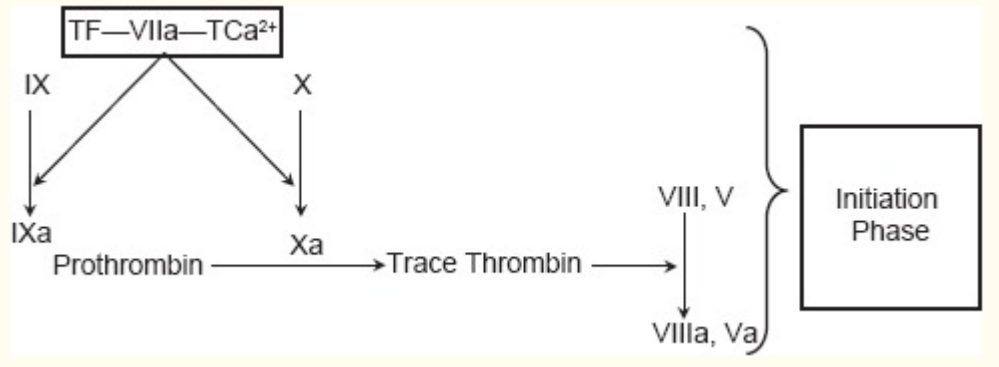
Activated factor X along with its cofactor (factor V), tissue phospholipids, platelet phospholipids and calcium forms the prothrombinase complex which converts prothrombin to thrombin.

The thrombin further cleaves circulating fibrinogen to insoluble fibrin. This also activates factor XIII, which covalently cross links fibrin polymers incorporated in the platelet plug. Fibrin network is created which stabilises the clot and also forms a definitive haemostatic plug [(3)].

Current Concepts of Coagulation:

It has been realized that the formation of the secondary hemostatic plug is much more complex than what was traditionally learnt as a simple cascade of events that are divided into extrinsic and intrinsic pathways. Current concepts show the following events to happen during the process. They are explained below in phases as Initiation, amplification, propagation and stabilisation phases [(6)].

a) **Initiation**



b) Amplification

Thrombin produced in initiation pathway,

- Activates factor V and factor VIII
- Act as a co factor in prothrombinase complex formation
- Accelerates the activation of Factor II by F Xa and also F Xa by F IXa

c) Propagation

Tenase complex and prothrombinase complex are accumulated on platelet surface which support continuous generation of thrombin and activation of platelets. This result in continuous thrombin generation and fibrin to form a large clot [(5)].

d) Stabilization

Thrombin generation

Activation of factor XIII (fibrin stabilizing factor) – convalently links fibrin polymers

Provides strength & stability to fibrin in platelet plug

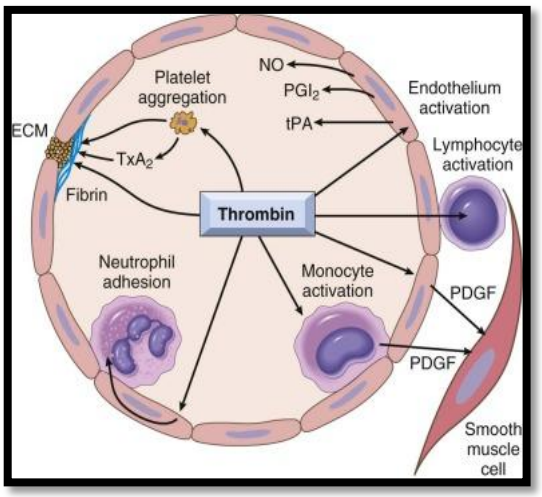
Thrombin activates thrombin activatable fibrinolysis inhibitor (TAFI) which protects the clot from fibrinolysis.

FUNCTIONS OF THROMBIN

Thrombin has variety of enzymatic activities which control various aspects of haemostasis. It also links coagulation to inflammation and repair [3].

Functions of thrombin include:

- Conversion of fibrinogen into fibrin cross links
- Activation of platelets
- Pro-inflammatory effects
- Anticoagulant



*(**Source of picture:** Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran. Pathologic basis of disease. 9th ed. Elsevier / Saunders; 2015).*

Activation of platelets

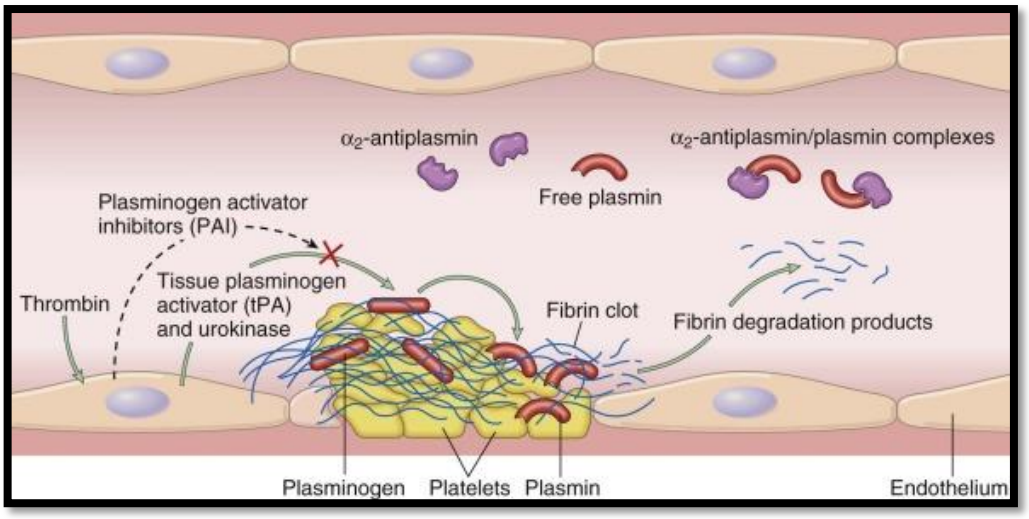
- Thrombin has the ability to activate PARs - act as a link between platelet function to coagulation
- Potent inducer of platelet activation and aggregation [3]

Pro-inflammatory effects

PARs are expressed on inflammatory cells and endothelium. Activation of receptors by thrombin contributes to tissue repair and angiogenesis

FACTORS LIMITING COAGULATION

Coagulation cascade activation leads to fibrinolytic cascade which limits clot size and causes clot dissolution. Fibrinolysis is achieved through plasmin which breaks fibrin & interferes with polymerization [3].



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Endothelium

The clot formation, propagation or dissolution is determined by the balance between anticoagulant and procoagulant activities of endothelium.The functions of the endothelium are

- Fibrinolytic effects
- Anticoagulant effects
- Platelet inhibitory effects

Fibrinolytic effects

t- PA synthesized by normal endothelial cells serve as an important component of fibrinolytic pathway

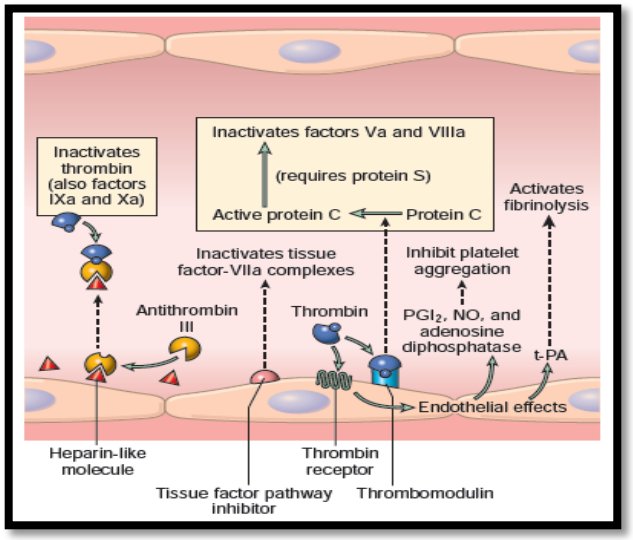
Platelet inhibitory effects

The adhesion of platelets to subendothelial collagen and VWF is prevented by the normal intact endothelium. The factors released by the normal endothelium prevent platelet activation and also aggregation. Nitric oxide, Prostacyclins (PGI2), adenosine diphosphatase are the few most important factors released by the endothelium. ADP is degraded by adenosine diphosphatase which act as an important activator of platelet aggregation. Thrombin activity is altered by the binding of endothelial cells. Thrombin also act as one of the potent platelet activator.[3,7]

Anticoagulant effects

Normal endothelium shields the vessel wall tissue factor from coagulation factors and also expresses many factors like thrombomodulin, heparin like molecules, endothelial protein C receptor and tissue factor pathway inhibitor. On the surface of endothelium, thrombomodulin binds thrombin and endothelial protein C receptor which binds protein C like a complex. If thrombin binds to this protein complex, it cleaves and activates protein C which requires a co factor called Protein S. This Protein C/ Protein S complex is an inhibitor of coagulation factors Va & VIIIa.

Heparin-like molecules present on the endothelial surface bind & activate antithrombin III.The antithrombin III inhibits factor IXa, Xa, XIa, and XIIa and also thrombin.



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Tissue factor pathway inhibitor needs protein S as a co factor and it inhibits tissue factor.

Test for Vascular and Platelet Phases

1). Bleeding Time: Although it is not done routinely now, when performed by standardized techniques (eg Ivy method), it is sensitive to detect platelet disorders (qualitative and quantitative) and collagen disorders (such as Ehlers Danlos Syndrome). It may be spuriously prolonged in hypofibrinogenemia and severe anemia.

2). Prothrombin time: The proteins involved in extrinsic pathway are FactorsVII, V, X and Fibrinogen. The function of these proteins can be assessed by Prothrombin time.

3). Partial thromboplastin time

The PTT access the function of proteins involved in intrinsic pathway. Factors XII, XI, IX, VII, X, V, II are involved in intrinsic pathway

DISORDERS OF PRIMARY HEMOSTASIS

CLINICAL MANIFESTATIONS OF BLEEDING DISORDER

- The clinical manifestations of bleeding disorder may vary from easy bruisability to life threatening hemorrhage. The severity of the

bleeding is proportional to the severity and type of the hemostatic defect whether it is primary or secondary.

- The type of bleeding can suggest the defective component of the hemostatic pathway. Bleeding from the skin or mucous membranes such as Epistaxis, Gingival bleed, menorrhagia indicates that the defect is in the primary hemostatic pathway components such as vessel wall and platelets.
- Secondary hemostatic pathway involves coagulation factors.Bleeding symptoms results from coagulation pathway abnormalities usually results in internal bleeding such as bleeding into joints and soft tissues.
- Bleeding from the subcutaneous blood vessels into the skin can be manifest as Petechiae, Purpura, Ecchymosis or Hematoma.
- Petechiae – small red to purple spots < 3mm in diameter. Usually occurs on the extremities due to high venous pressure. Petechiae are characteristic of blood vessel wall and platelet abnormalities and are not seen in coagulation factor abnormalities.
- Ecchymosis – bruises > 1 cm in diameter, produced when blood escapes into subcutaneous tissue. Blood vessels, platelets or coagulation factor abnormalities together can results in ecchymosis.
- Purpura – Intermediate lesions of size > 3mm but < 1 cm.

- Easy bruisability – If Ecchymosis & Purpura are found in high numbers but less than in trauma.
- Hematoma- can occur in any organ and it occurs due to leakage of blood from vessel wall opening and the blood gets collected beneath the intact skin forming blue or purple raised area [(7).]

DISORDERS OF THE VASCULAR SYSTEM

Disease of the vascular system can be Hereditary or acquired.Hereditary vascular disorders results from abnormal synthesis of subendothelial connective tissue or extracellular matrix components [(7)].

Characteristics of Hereditary disorders of connective tissue

Disorder	Features	Laboratory findings
Hereditary hemorrhagic telangiectasia (HHT	Arteriovenous malformations	Usually Normal
EhlerDanlos syndrome	Fragility of bloodvessels and tissues joint hypermobility & instability	BT - increased
Marfan Syndrome	Arachnodactyly,hypermobility of the joints, decreased tensile strength	Usually Normal
Osteogenesisimperfecta	Patch,defective bone matrix,brittle bones which cause easy fracture	Usually Normal
Pseudoxanthoma elastucum	Degeneration and calcification of elastic fibres	Usually Normal

Acquired disorders of vascular system

Disorder	Examples
Purpura resulting from decreased connective tissue	Senile purpura Scurvy Cushing syndrome & Glucocorticoid therapy
Purpura resulting from vasculitis	Henoch – SchonleinPurpura Infections Drugs
Purpura associated with dysproteinemias	Paraproteins (cryoglobulinemia,cryofibrinogenemia) Amyloidosis
Miscellaneous causes	Artificially induced purpura Mechanical purpura Purpurafulminans Easy bruising syndrome

PLATELET DISORDERS

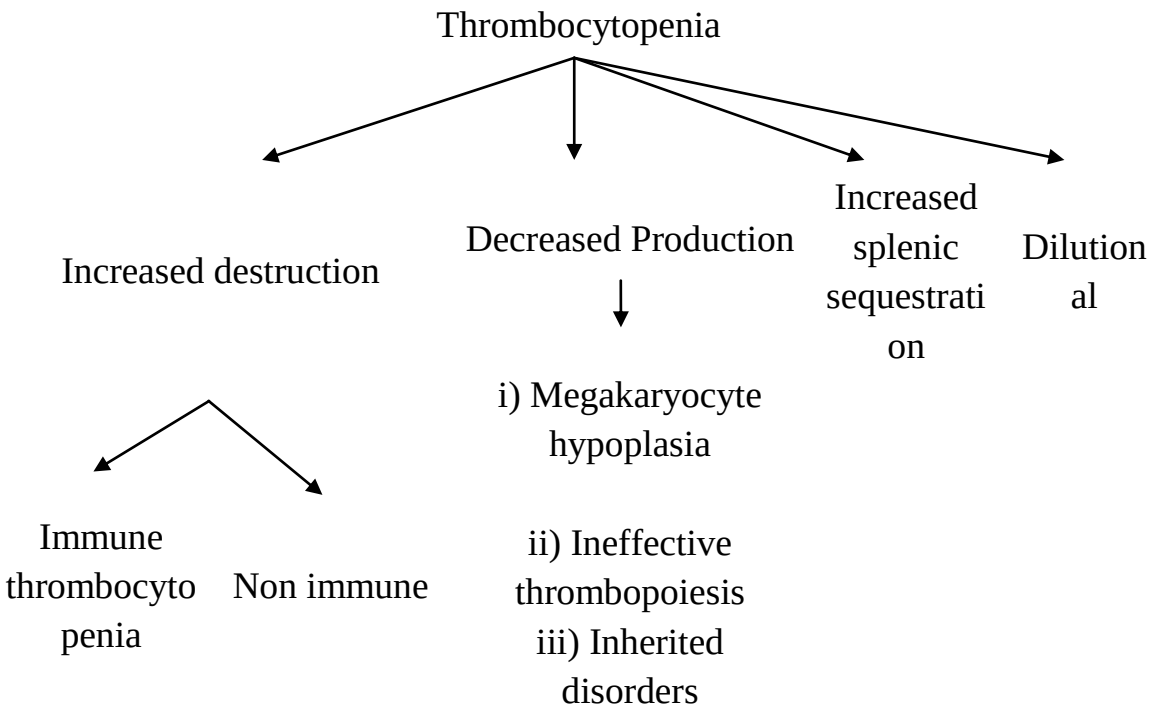
- Platelets play a main role in hemostasis. It maintains the integrity of the blood vessels and results in formation of primary hemostatic plug in response to injury.
- Primary hemostatic plug formation needs adequate normal functioning platelets. Decrease in number of platelets (Thrombocytopenia) or abnormal function of platelets (Thrombocytopathy) results in excessive bleeding [(8)].
- Platelet disorders are of two types – Quantitative and Qualitative. In quantitative disorders, the platelet count is below

(thrombocytopenia) or above (thrombocytosis) the reference interval. Qualitative disorders show platelet function abnormality.

Quantitative Platelet disorders

Quantitative platelet abnormalities include thrombocytopenia or thrombocytosis. The reference interval of platelet count is 150 to 400×10^9 / L. Platelets are counted by automated instruments.

Classification of Quantitative Platelet Abnormalities [7]



Thrombocytosis

- Primary thrombocytosis
- Secondary thrombocytosis
- Transient thrombocytosis

Artifacts in the quantitative measurements of Platelets

Qualitative (Functional) Platelet disorders

- Hereditary
- Acquired

Inherited disorders of Platelet function

Disorders of Platelet adhesion

- Von Willebrand disease (Deficiency/defect in plasma VWF)
- Bernard Soulier syndrome (Deficiency/defect in GPIb/IX)
- Deficiency/defect of collagen receptors(GPIa / IIa , GPVI)

Disorders of aggregation (Defects in platelet –platelet interaction)

- Congenital afibrinogenemia (Deficiency of platelet fibrinogen)
- Glanzmann Thrombosthenia (deficiency /defect in GPIIb / IIIa)

Disorders of platelet secretion and abnormalities of granules

- Storage pool deficiency (α-SPD, δ-SPD)
- Quebec platelet disorder

Disorders of platelet secretion and signal transduction

- Receptor defects (defects in platelet – agonist interaction)
- Defects in G protein activation

- Defects in Phosphotidylinositol metabolism (phospholipase C deficiency)
- Defects in protein phosphorylation (PKC deficiency)
- Abnormalities in arachidonic acid pathways & TXA2 synthesis (deficiency of phospholipase A2,cyclooxygenase, thromboxane synthesis)

Disorders of platelet coagulant – protein interaction

Scott syndrome

Disorders of Platelet adhesion

Primary hemostasis is initiated when platelets are adhered to exposed subendothelium. Adequate functional VWF and functional GPIb/IX on platelet membrane are essential for adhesion. VWF acts as a bridge between binding of platelet via GPIb /IX and to collagen. Deficiencies of either GPIb/IX or vWF results in defective platelet adhesion [(9)].

Bernard Soulier syndrome:

This is an autosomal recessive disorder. Bleeding symptoms are usually manifest soon after birth or in early childhood. Clinical manifestations can be epistaxis, purpura, gingival bleed, menorrhagia or rarely gastrointestinal bleed, hematuria or hematomas. Trauma, major surgical procedures can

result in severe bleeding episodes. The severity and frequency of bleeding vary between individuals [9].

Laboratory investigations

- Increased Bleeding time
- Mild thrombocytopenia
- Peripheral Smear showing Giant Platelets with diameter as high as 10 μm
- Platelet aggregometry shows defective platelet aggregation to ristocetin

The diagnosis of Bernard Soulier syndrome requires the following

- Macro thrombocytopenia
- Increased bleeding time
- Defective agglutination to ristocetin
- Flow cytometry – Low or absent levels of platelet GPIb-V-IX (CD42a d)

Treatment

- General and specific treatment of bleeding and antiplatelet agents like aspirin can be given
- Desmopressin and rFVIIa administration is also effective in some patients [8, 9].

Von Willibrand disease (VWD)

- VWD was first described by Erik von Willebrand in 1925 [10].
- Quantitative or qualitative deficiency of Von Willebrand factor arising from mutations in VWF gene.

VWF is a large multimeric glycoprotein which serves as a carrier protein for factor VIII and causes platelet adhesion to damaged endothelium

Deficiency of VWF leads to mucocutaneous and posttraumatic bleeding.

Markedly reduced or absent VWF leads to secondary decrease in factor VIII which cause muscle or joint bleeds

Pathogenesis

- VWF is a large multimeric glycoprotein which is synthesised in megakaryocytes and platelets and stored in alpha granules in platelets and Weibel-Palade body in endothelial cells.
- After injury to blood vessel wall endothelium, VWF binds with sub endothelial collagen. After binding of VWF to exposed sub endothelial collagen there is a conformational change that causes binding to platelet glycoprotein Ib-IX-V complex (GPIb-IX-V) receptor results in platelet adhesion to damaged endothelium. VWF also binds with the αIIbβIII (GPIIb IIIa) receptor on platelets [7].

- VWF also serves as a carrier protein for plasma factor VIII.

Classification: Depends on quantitative or qualitative defect in VWF, VWD has three major categories

- Type 1 (Quantitative defect of VWF)
- Type 2
- Type 3 (Quantitative defect of VWF)

Type 2 VWD is characterised by Qualitative abnormalities of VWF and is further subdivided into types 2A, 2B, 2M, 2N [10, 11].

Types of Von Willebrand disease

Type	Description	% of VWD
1	Partial quantitative deficiency with normal structure & functions of the multimers	70 – 80
2	Qualitative disorder with functionally abnormal VWF	
2A	Decreased platelet adhesion; absence of largest multimers	10 to 15%
2B	Increased affinity for platelet GPIb, absence of largest multimers	< 5%
2M	Decreased platelet adhesion not due to absence of largest multimers	Rare
2N	Decreased affinity for FVIII (AR)	Rare
3	Absence of VWF in platelets and plasma	0.5 – 5 /million

Clinical manifestations are primarily epistaxis, ecchymosis, menorrhagia, and postoperative or postpartum bleeding [12].

Laboratory investigations include

- Von Willebrand Factor Antigen assay by ELISA
- Ristocetin Cofactor Activity Assay - Ristocetin induces binding of VWF to the GPIb receptor on platelets
- Collagen-Binding Assay

Desmopressin is useful in the treatment of vWD.

Disorders of Platelet aggregation

Platelet aggregation requires fibrinogen and presence of GPIIb /IIIa receptor on platelet membrane. The congenital defect in GPIIb/IIIa receptor results in Glanzmann Thrombasthenia.

Glanzmann Thrombasthenia (GT) is an autosomal recessive disorder, also known as the integrin $\alpha_{iib}\beta_{3.}$

Glanzmann Thrombasthenia is due to Quantitative or qualitative abnormalities of the platelet GPIIb- GPIIIa, characterised by defective in vitro platelet aggregation.

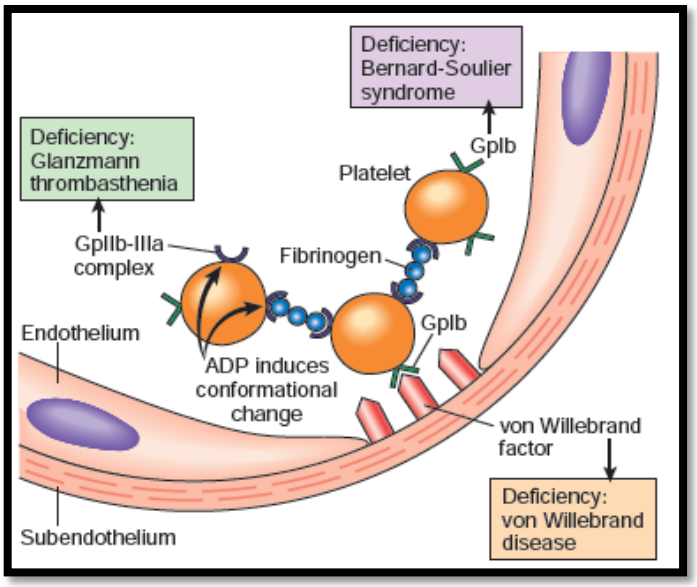
Types

- Type 1 - Undetectable or trace amounts (<5%) of GPIIb/IIIa , absence of αG fibrinogen .

- Type 2 - GPIIb /IIIa – 10 to 20 %, αG fibrinogen present.
- Type 3 - Qualitative defect of GPIIb/IIIa, levels of GPIIb/IIIa are 50 to 100% [15].

Pathogenesis

GPIIb-IIIa is a calcium-dependent heterodimer which binds to fibrinogen or vWF and also to fibronectin. The genes for GPIIb and GPIIIa are located in the long arm of chromosome 17 (17q21-22). Genetic defect in either GPIIb or GPIIIa will inhibit the synthesis of GPIIb-IIIa complex and also prevents the assembly of the receptor. This leads to lack of fibrinogen receptor. After platelet activation the binding of fibrinogen is deficient, leads to defective or absent platelet aggregation [11].



Source of picture: *Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran. Pathologic basis of disease. 9th ed. Elsevier / Saunders; 2015).*

Clinical Features

- Hemorrhagic symptoms occur only in patients who are homozygous for GT mutations and heterozygous condition is asymptomatic mostly. Hemorrhagic symptoms can be epistaxis, gingival hemorrhage, and menorrhagia
- Gastrointestinal bleeding and hematuria are less common

Laboratory findings include

- Prolonged bleeding time
- Deficient clot retraction
- Deficient platelet aggregation with ADP, collagen, epinephrine or thrombin
- Ristocetin-induced aggregation and coagulation tests are normal

Treatment

- Topical thrombin and antifibrinolytic agents (tranexamic acid) are useful in localized bleeding
- Nasal packing is done for epistaxis and gingival bleeding.
- Recombinant factor VII a (rFVIIa) is helpful in patients developed antibodies or with history of transfusion refractoriness [(8)].

Disorders of platelet secretion and abnormalities of granules

Group of congenital disorders have deficiency of granules and their constituents. So there is defective ADP release from activated platelets and abnormal secretion dependent platelet aggregation.

Defective platelet secretion can occur due to either absence or defect in one or both dominant types of platelet granules.

- Isolated deficiency of α-granules (α-SPD) - Gray platelet syndrome
- Abnormality of dense granules - (δ-SPD)
- Abnormalities of both α- and δ-granules - αδ-SPD

α-Granule Storage Pool Disease: Gray Platelet Syndrome

It is a very rare disorder, till now less than 100 cases have been reported worldwide and it is an autosomal trait with mild history of bleeding.

Pathogenesis

In this disorder there is selective deficiency of the content and number of α-granules. Though the formation of these granules in megakaryocytes occurs normal but the granule number decreases during maturation. Finally the megakaryocytes have only few small and abnormal granules. Few normal protein components of α-granule membranes like GPIV, integrin $\alpha_{IIb}\beta_3$integrin and P -selectin are persist in the abnormal granules.

During platelet activation these normal protein constituents are normally redistributed and the defect is limited to Megakaryocytes and platelets. Some proteins are synthesised but not properly stored in abnormal α-granules. δ-granules contain normal serotonin and adenine nucleotides [(11)].

Laboratory findings

- Prolonged bleeding time
- Decreased platelet count (less than 50,000/μl)
- Peripheral smear shows large misshaped agranular gray platelets

Platelet aggregation study shows Normal aggregation and release to arachidonic acid and variable aggregation by ADP, epinephrine, collagen or thrombin.

Desmopressin (DDAVP) shortens the bleeding time in some patients.

Isolated δ-Storage Pool Diseaseis an autosomal dominant disease. Patients presents with mucocutaneous bleeding, easy bruising and excessive postoperative bleeding.

Laboratory findings are

- Normal platelet count
- Prolonged bleeding time

- Platelet aggregation to ristocetin is normal and show impaired response to collagen.

Management: Desmopressin (DDAVP) and antifibrinolytics such as tranexamic acid are useful.

Combined αδ-Storage Pool Disease is less common than isolated δ-SPD, is an autosomal dominant disorder. Combined αδ is characterised by Uniform decrease in δ-granules and contents and variable deficiency of α-granules and/or their constituents.

DISORDERS OF SECONDARY HEMOSTASIS

Deficiencies of coagulation factor abnormalities lead to large joint bleeds.

1) Hemophilia A (Classic haemophilia, Factor VIII deficiency)
2) Hemophilia B (Factor IX deficiency)
3) Factor II deficiency
4) Factor V deficiency
5) Combined Factor V and VIII deficiency
6) Factor VII deficiency
7) Factor X deficiency
8) Factor XI deficiency
9) Factor XIII deficiency

Haemophilia A

Haemophilia A is the most common inherited coagulation factor abnormality and is an X linked recessive disorder caused by defective synthesis of factor VIII [13].

Pathogenesis

In coagulation pathway, the activated factor IX (IXa) complexes with calcium, activated factor VIII (VIIIa) and phosphatidylserine on physiological membranes to produce Factor Xa which then involved in formation of prothrombinase complex. Activation of factor X needs factor VIII and Factor IX for normal thrombin generation. In case of deficiency of these proteins result in inadequate or defective generation of thrombin and fibrin. Thrombin is essential for platelet aggregation, Factor VIII activation and clot retraction.

Clinical manifestations can be

- Recurrent hemarthrosis is characteristic. Excess bleeding into various tissues includes Hemarthrosis and soft tissue hematomas. Hemarthrosis sometimes leads to severe crippling hemarthropathy.
- Mucous membrane bleeding

- Gastrointestinal bleeding – associated with older age & complications of advanced liver disease
- Pseudotumors (Blood cyst *)* – Rare and dangerous complication
- Neurologic complications
- Intracranial bleeding
    - Dangerous hemorrhagic complications
    - Leading cause of death in hemophiliac patients

Clinical classification of Hemophilia [11,12]

Classification	Factor VIII level	Clinical features
Mild	6 to 40% of normal	Rare spontaneous bleed Hemorrhage secondary to trauma/ surgery
Moderate	1 to 5% of normal	Bleeding at circumcision Excessive spontaneous bleed after surgery/trauma
Severe	≤ 1% of normal	Spontaneous hemarthrosis with crippling – frequent Frequent severe spontaneous hemorrhage

Laboratory investigations

- Prolonged activated partial thromboplastin time (aPTT)
- Normal Prothrombin time
- Mixing studies – corrected with equal volume of normal plasma
- Factor VIII assay – decreased factor VIII

Management

- Factor VIII replacement therapy
- Desmopressin is a synthetic vasopressin analogue that increases plasma factor VIII and VWF levels.
- Antifibrinolytics such as (traxenamic acid, €- aminocaproic acid (EACA) are useful in controlling bleeding [8,9].

Factor IX deficiency (Hemophilia B)

Hemophilia B is a X linked recessive disorder and occurs due to deficiency of factor IX.

Pathogenesis

- Factor IX is a vitamin K dependent glycoprotein. Factor IX is activated by tissue factor and factor VII a complex. After activation, the activated factor IXa in the presence of calcium, phospholipids along with factor VIIIa further activates factor X. Factor Xa converts prothrombin to thrombin which also requires calcium, factor Va and activated platelets.
- The absence or dysfunction of factor IX results in Hemophilia B. The clinical severity of this disease is correlated with factor IX functional activity [16].

Clinical features

Bleeding manifestations similar like Hemophilia A but are less severe complications than haemophilia A.

Laboratory features

- Prolonged aPTT
- Prothrombin time - normal
- Factor IX assay

Management: Replacement of factor IXb can be administered.

Fibrinogen deficiency

Fibrinogen deficiency is one of the rare coagulation factor abnormality due to defect in quantitative or qualitative or both. Quantitative deficiency of fibrinogen can be either hypofibrinogenemia which is characterised by fibrinogen levels less than 1.5 g/dl or afibrinogenemia due to complete absence of fibrinogen. Qualitative deficiency is due to functional defect of fibrinogen. Both quantitative and qualitative fibrinogen deficiency is called as hypo dysfibrinogenemia [(17)].

Patients with afibrinogenemia have variable severity of bleeding symptoms include bleeding from umbilical cord, mucosa, gastrointestinal, genitourinary or central nervous system. First trimester abortion is also

common. Patients with hypofibrogenemia have mild bleeding symptoms. The bleeding symptoms in dysfibrinogenemia is unpredictable, hemorrhages usually occur after trauma [18].

Factor II deficiency

One of the rarest coagulation factor deficiencies, it is an autosomal recessive disorder.

- Prothrombin deficiency can be either quantitative or qualitative
- Type 1- Hypoprothrombinemia (True deficiency)
- Type 2- Dysprothrombinemia- due to dysfunction of Prothrombin [19]

Types

- Type 1 deficiency is a quantitative defect , characterised by low or unmeasurable antigen levels.
- Type 2 deficiency is a qualitative defect with normal or mildly decreased antigen levels.[14](Prothrombin deficiency can be classified based on the blood levels of prothrombin as
- Mild - >10% prothrombin levels in blood
- Moderate - 5 to 10%
- Severe - < 5%

Heterozygotes remain asymptomatic. In severe prothrombin deficiency there is marked bleeding [20].

Laboratory investigations

- Prolonged PT
- Mild increase in APTT
- Normal TT

Management

- FFP
- Prothrombin complex concentrates (PCCs)
- Replacement therapy is essential in case of severe prothrombin deficiency.

Factor V deficiency

Inherited factor V deficiency was initially called as parahemophilia and is an autosomal recessive disorder.Factor V, also known as labile factor or proaccelerin, is a vital cofactor of the prothrombinase complex.

Clinical manifestations

Factor V deficiency can be classified as

- Mild - ≥ 10 % of factor V levels
- Moderate - < 10%
- Severe – undetectable factor V levels [21]

Plasma factor V activity is 25 to 60% of normal in heterozygotes who are asymptomatic

Common symptoms include epistaxis, ecchymosis, mucosal bleed and menorrhagia and the laboratory tests show Prolonged PT& APTT [21]

As Factor V immunoassay is not available readily, the functional deficiency of factor V can be identified by clotting assays. Factor V deficiency can be managed with FFP.

Combined Factor V and VIII deficiency

Autosomal recessive disorder characterised by deficiencies of both factor V and factor VIII ,concomitantly low but detectable factor activity and antigen levels (5 to 20%) [21].

Pathogenesis: Mutations responsible this combined factor V and VIII deficiency are located in the

- LMAN1 gene on chromosome 18q21.32. LMAN1 (Lectin mannose binding 1) –acts as a molecular chaperon in the synthesis of factor V and VIII

Mutations in MCFD2 (multiple coagulation factor deficiency)gene which acts as a cofactor in the intracellular trafficking of both FV and FVIII [21].

Clinical manifestations range from mild to moderate bleeding symptoms.

Management: Depends on the severity of bleeding, FFP can be administered [9].

Factor VII deficiency

Factor VII deficiency is one of the most common rare congenital bleeding disorder. It is an autosomal recessive disorder [22].

Clinical manifestations

Inherited FVII deficiency has a wide range of clinical manifestations, varies from most frequent mild mucocutaneous bleeding to10 to 15% of life threatening hemorrhages (CNS or Gastrointestinal) [22].

Bleeding occurs in homozygous mostly. Heterozygous remain asymptomatic. Clinical heterogeneity is the typical feature. Most common symptoms include menorrhagia and epistaxis. Neonates with factor VII deficiency have increased risk for intracranial bleeding during delivery.

Types: Factor VII is divided into two groups.

Type 1 - Quantitative defects characterised by decrease in both Factor VII activity and antigen levels.

Type 2 is characterised by decreased factor activity and with normal antigen levels [23].

Lab investigations

- Prolonged PT
- Normal PTT
- Normal TT
- Factor assay- factor VII antigen assay is determined by ELISA or immune turbidimetric assays.

Factor VII assay helps to distinguish between type 1 and type 2 defects [22]

Management: For acute bleeding, most widely accepted is Recombinant FVIIa replacement therapy.

Fresh frozen plasma (FFP, PCC) can also be used [24]

Factor X deficiency is a very rare autosomal disorder

- Type 1 – Both Antigenic & functional activity of factor X are decreased.
- Type 2 – Functional activity is decreased but antigenic levels are normal.

Clinical features are related to functional levels of the protein. Bleeding may occur at any age. The most common bleeding symptoms are epistaxis, and menorrhagia [25].

Patients with severe factor X deficiency can present with umbilical stump bleeding in neonatal period [26].

If Factor X activity is < 10 % of normal there is high incidence of major bleeding.

Lab investigations

Prolonged PT and APTT

Factor X functional assay and immunologic assays

Factor X deficiency is managed by PCC, FFP [27]

Factor XI deficiency

- Factor XI deficiency is an autosomal recessive disorder .There is high prevalence of factor XI deficiency in Jewish persons.
- Factor XI deficiency is associated with other inherited coagulation disorders such as Von Willibrand disease, factor VIII deficiency and factor VII deficiency. These associations are common in Jewish population because of the high prevalence of factor XI deficiency. Bleeding manifestation is rare. Bleeding always occur during insult such as trauma, surgery [11].

Lab investigations

- Prolonged APTT

- Normal PT

Factor XIII deficiency

Factor XIII (Fibrin Stabilising factor) cause stabilization of the clot and coss linking of fibrin polymers thus prevents from fibrinolysis

Clinical manifestations can be any of the following [27].

Delayed umbilical bleeding

Neonate – delayed separation of umbilical cord stump.

Poor wound healing

Recurrent spontaneous hemorrhage

Based on deficiency of antigen and dysfunctional protein factor XIII deficiency classified into two types

- Type 1 is due to Antigen deficiency
- Type 2 –is due to Dysfunctional protein

Laboratory investigation

- Normal coagulation screening tests (BT,CT,APTT,PT)
- If patients with clinical symptoms of bleeding manifestations but the coagulation screening tess are normal it is necessary to do factor XIII assay to rule out the underlying bleeding disorder

Management

- FFP
- Cryoprecipitate - One bag of cryoprecipitate contains 75 units of factor XIII
- Lyophilized heat treated plasma derived factor XIII concentrate is available

Contact Factor Deficiencies

- Deficiencies ofcontact factors such as factor XII, prekallikrein, and high-molecular-weight kininogen which can be manifest as bleeding symptoms are extremely rare although factor XI deficiency is associated with bleeding.These contact factors play a role in initial steps of intrinsic pathway of coagulation.
- Contact factors level < 1% of normal results in much prolongation of APTT than Hemophilia A or B [(9).]

Multiple factor deficiencies

Hereditary multiple factor deficiencies are rare disorders with variable severity of bleeding disorders. Combined deficiency of Vitamin K dependent clotting factors (factor II ,VII, IX ,X)result from defective vit K metabolism or defective vit K dependent carboxylation [(9)].

REVIEW OF OCCURRENCE OF HEREDITARY / CONGENITAL HEMOSTASIS ACROSS VARIOUS STUDIES

According to the Oxford Monograph on Medical genetics, Vol.no 5, the most common congenital bleeding disorders in the general population were VWD, Haemophilias A&B and Factor XI deficiency. This information was published in 1994, after an extensive data base survey of all the haematology clinics in UK [(28)].

In 2004, Acharya SS, Coughlin A, Dimichele DM et al published the findings of the North American Registry for rare bleeding disorders. The findings were the outcome of a large survey conducted in 1999 by sending a questionnaire to the American &Canadian Hemophilia treatment centres. Apart from basic demographic information they also sought information on Family history, clinical manifestations, treatment and therapy related complications. Based on factor levels of coagulation factors II, VII, X and V, they classified the patients as homozygous (level<0.2/mL) or heterozygous (levels ≥0.2/mL). Similarly those two who had Fibrinogen levels less than 50 mg /dl were considered homozygous while those who had values between 50mg/dl and lower limit of reference range were considered heterozygous.

The age at diagnosis varied from birth to 73 years with a median age of 7 years.50% of the respondents had at least 1 family member affected. Of the all rare bleeding disorders, deficiency of factor VII was the most common (46% of all). About 3% had combined deficiencies where the common denominator was factor VII with a combination of factor V, X or II. Combinations such as Factor VIII &V, Factor V, VII& X were extremely rare. (ie. one of each)

Most of the affected patients were Caucasians except for factor II deficiency which was most common in the Latinos.

Factor II deficiency was inherited most often more homozygous than heterozygous with a median factor activity of 0.03U/ml. In 60% of both the homozygous and heterozygous population, the bleeding was unprovoked while in 40% it was trauma induced. APCC or non activated PCC was used for treatment in most patients. A few others received FFP or EACA alone.

Factor VII deficiency was found more commonly in heterozygous state (64%) than homozygous. Most cases were diagnosed following an abnormal PT, while the remainder were diagnosed after spontaneous bleeding.

Most of the haemorrhage was into the skin and mucous membranes. Treatment received ranged from PCCs, Factor VIII concentrates, FFP or EACA.

Factor X deficiency was more often in homozygous patients (56%) and the most common diagnostic event was non surgical bleeding that was predominantly into the skin and mucous membranes. Most patients were treated either with PCC or FFP.

Factor V deficiency had an equal distribution between hetero and homozygous. Presence of abnormal bleeding into skin/mucous membrane or an abnormal perioperative lab screen led to the diagnosis in most patients.

Factor XIII deficiency manifested as spontaneous haemorrhage either at umbilical stump or into joints, muscles, genitourinary tract etc. The response to SDFFP or CPP and EACA was good.

Hypo and dysfibrinogenemia had an equal occurrence followed by afibrinogenemia amongst those with fibrinogen disorders. Nonsurgical or post operative bleeding and a positive family history were the most common diagnostic events. In all the three types, bleeding was more often following a challenge rather than spontaneous.

Of these rare bleeding disorders, the most common disease complication was anaemia followed by musculoskeletal complications [(29)].

In a review article published in NEJM in 2001, it was obvious that of all the inherited deficiencies of factors of coagulation associated with abnormal bleeding, the most common were deficiencies of Factor VIII (1 in 10,000),

Factor IX (1 in 60,000) and factor VII (1 in 5 lakhs).The remainder occurred in a frequency of 1 in 1 million to 1 in 2 million. The incidence of haemophilia A in male live births was 1 in 5000 while that of haemophilia B it was 1 in 30,000. However, deficiency or dysfunction of VWF was the most frequent bleeding disorder (1 in 1000). The haemophilias were classified as severe, moderate and mild based on factor activity i.e., ≤ 1%, 2to 5% and 6 to 30% respectively [(30)].

In 2005, a study on the spectrum of Inherited bleeding disorders in the Indian population was published from AIIMS, Delhi. They consolidated all the records between the period 1998 and 2002 to obtain the data. The haematology lab tested for the factor deficiencies by one-stage method. While platelet aggregation studies using agonists such as ADP, epinephrine, arachidonic acid, collagen and ristocetin were performed on a platelet aggregometer called Chronolog. They observed that of the 966 patients, 586(60.6%) had inherited defects in coagulation, followed by Platelet function defects. Among the former, factor VIII deficiency was the commonest (42.4%) followed by VWD(8.5%) and factor IX (5.1%). Deficiencies of coagulation factors II, XI and XII were rare.

Haemophilia A&B occurred in a ratio of 8:1 and 63% of them were severe haemophilias with <1% of factor activity. Only 3 of the 459 cases occurred

in females. VWD was the most common coagulation defect in females followed by deficiencies of factor X and XIII.

The age range for the inherited disorders was birth to 35 years with a median of 7.2 years. 17.8% of patients with haemophilia and 21.9% of patients with VWD had a positive family history.

Haemarthrosis was the most common clinical features in the haemophilias. Females with vWD presented most often with menorrhagia. Factor XIII deficiency presented most often as CNS bleeding.

Of the inherited platelet function defects, the most common was isolated PF3 availability defect followed by unclassified defects and Glanzman's thrombasthenia. Most of these patients (89.6%) presented with mucocutaneous bleeding only and presented after 25 years of life [(31)].

Another publication from AIIMS which reviewed results of abnormal bleeding between the period 2001 and 2005 showed similar results. During this period, 576 patients were diagnosed to have an inherited bleeding disorder. Haemophilia A was the commonest (52.3%) followed by vWD and Haemophilia B. Of these haemophilia A patients had the most severe bleeding manifestation. Amongst the platelet function defects, isolated PF3 availability defect was the most common. The main difference this study showed in comparison with their previous study was that vWD had an

almost equal sex incidence. Type 2 VWD was more common (53.67%) than types 1 or 3 VWD [32].

In 2009, Bhushan Asthana, Prashant Sharma, Ravi Ranjan et al from AIIMS, Delhi published their findings on bleeding disorders for the year 2006.They noted that 77.4% (1040 cases)had an underlying acquired bleeding disorder of which DIC was the most common (28.5%) followed by deranged liver function, neurosurgical causes, cancers etc. About 22.6% (302cases) were diagnosed to have congenital / inherited cause for the bleeding disorder of which 201 cases were due to coagulation factor defects and 101 were due to inherited platelet disorder. Haemophilia A and isolated PF3 availability defect were the leading causes in the two groups, respectively. The most common causes for DIC were septicemia, pneumonia, liver and renal diseases .Road traffic accidents accounts for 21% of DICs [33].

In 2004, two researchers from Italy investigated patients from Italy and Iran who suffered from recessively inherited coagulation disorders. They chose Iran because of the very high rate of consanguineous marriages and high incidence of recessive diseases. For the same study period, Iran had twice the number of cases than Italy. In both countries, factor VIII deficiency was the most common. When non –X linked recessive diseases were analysed, the top three diseases in Iran were deficiencies of factor VII, factor XIII and

combined Factor V & III deficiency and fibrinogen deficiency. In Italy, while factor VII was the first most common, the second and third places were due to factors XI and XIII deficiencies respectively. Patients with deficiencies of Fibrinogen , Prothrombin, factor X , factor XIII and vitamin K dependent multiple deficiency presented with umbilical stump bleeding, while mucosal tract bleeding was the primary manifestation in patients with deficiencies of factors V, VII. Factor XI deficiency presented only as posttraumatic bleeding [(34)].

A study from Iraq published in 2010 showed that over 90% of cases of inherited bleeding disorders were either Haemophilia A or VWD. 82.7% patients had their clinical symptoms during the first year of life itself. Haemarthrosis was seen most often with Haemophilia followed by factor X deficiency. About 50% of these patients had limitations of three or more joints [(35)].

In 2012, researchers from AIIMS Delhi published their findings on rare inherited coagulation disorders. They noted that 43% were due to factor X deficiency followed by deficiencies of factors XIII and VII. It was noted that during a similar period, factor VII deficiency was the most common rare inherited disorders of coagulation in the North Americans and Iranians. Almost all of these deficiencies presented primarily as mucocutaneous bleeding [(36)].

In 2012, Peyvandi F, Palla R, Menegatti M et al published the results from the European Network of Rare coagulation disorders. On a consensus, the bleeding severity was classified as shown below.

S No	Clinical bleeding severity	Definition
1	Asymptomatic	No documented bleeding episodes
2	Grade I bleeding	Bleeding that occurred after trauma or drug ingestion (antiplatelet / anticoagulant therapy)
3	Grade II bleeding	Spontaneous minor bleeding such as bruising, epistaxis, oral cavity bleeding ,menorrhagia etc.
4	Grade III bleeding	Spontaneous major bleeding such as intramuscular hematomas requiring hospitalization, hemarthrosis, CNS/GI/Umbilical cord bleeding.

They observed that of the 592 patients with rare bleeding disorders, the age range was 7 months to 95 years with a mean age of 31 years. Maleto female incidence was almost equal. The most common factor deficiencies were factor VII (38%) and factor XI (22%). 45.8 % were asymptomatic, while patients with Grade I, II, III bleeding accounted for 17.8%, 23.9% and 12.5% respectively. On linear regression analysis, there was a direct correlation between factor activity and severity of bleeding for fibrinogen, combined factor V and VIII, X and XIII but not for factor V and VII [(37)].

A study from CMC Vellore showed that in South India, Factor XIII was the most common of rare bleeding disorders, while factor XI deficiency was the rarest. Correlation of severity of bleeding and factor activity assays was noted only with factor VII and X deficiencies [(38)].

Roberta Palla et al noted that after all the Haemophilias an vWD, the common causes for rare bleeding disorders were deficiencies of factors VII and XI. Factor XIII deficiency was to be suspected when a congenital bleeding disorder presented with normal results for PT, APTT, and TT. For all other deficiencies any one or more of these tests showed abnormal results [(20)].

In 2016, a study conducted at JIPMER, Pondicherry was published. They had 32 patients treated in the Paediatrics Department for bleeding manifestations. They excluded acquired bleeding disorders and observed demographic details, results of investigations and clinical manifestations. 81.28% of these patients had clotting disorders while the remainder had platelet disorders.56.3% of clotting disorders was due to Haemophilia A followed by Haemophilia B (9.4%). Other causes include deficiencies of factors XIII, VII and Fibrinogen. Of the six numbers of platelet disorders, Bernard Soulier syndrome accounted for 2 cases and the remainder 4 were labelled as Thrombasthenia. Of the 26 clotting disorders, 23 occurred in males and 9 had a positive family history. Most of the Haemophiliacs

presented under less than one year of age. Haemophilia A patients presented more commonly with subcutaneous hematoma followed by Haemarthrosis and bleeding gums. Haemarthrosis was most commonly noted in knee joint followed by Elbow joint [39].

A review article on inherited bleeding disorders in the genomic era summarized the differences between coagulation factor defects and platelet disorders based on history, as in the following table.

Findings	Coagulation factor defect	VWD/Platelet disorder
Onset of bleeding	Delay after trauma	Spontaneously or immediately after trauma
Mucosal bleeding & Petechiae	Rare	Common /characteristic
Ecchymoses	Large & Solitary	Small & multiple
Haemarthrosis	Characteristic	Rare
Bleeding from small cut	Minimal	Persistent
Gender	80 – 90 % males	Equal incidence

At least 20 tier 1 genes that are associated with Platelet disorders are approved by the International society on Thrombosis& Hemostasis. There are about 30 more tier 2 genes in the pipeline. These genetic diagnosis may be made by any one or more of these platforms ie, Whole genome expression assays, Next Generation Sequencing and genome wide association studies [40].

In the most recent article published in 2019, Menegatti M and Palla R presented the summary of lab diagnosis of rare coagulation disorders as shown in the table.

Deficiency	APTT	PT	TT
Afibrinogenemia	↑↑	↑↑	↑↑
Dys /Hypofibrinogenemia	↑	↑	↑↑
Prothrombin	↑	↑	N
Factor V	↑	↑	N
Combined Factor V&VIII	↑	↑	N
Factor VII	N	↑	N
Factor X	↑	↑	N
Factor XI	↑	N	N
Factor XIII	N	N	N
Vit K dependent coagulation factors	↑	↑↑	N

The article also stresses on stringent IQC and EQA for each of the parameters. Certified reference materials need to be used for assay standardization. They also stress that for assessing factor XIII activity,

solubility screen must not be the sole criterion, due to false positives. Hence factor XIII assays need to be done in all suspected cases of abnormal bleeding with normal PT, APTT and TT [41].

This is a case series study, observational in nature and involved both retrospective and prospective study of data of patients who presented to Clinical Pathology Laboratory for Investigation of Abnormal Bleeding.Most cases are referred from other hospitals for investigation of hemostatic work up. Of the diagnoses offered, cases suggestive of congenital bleeding disorders were identified and the Clinicopathological profile was analyzed.The study wascommenced after getting the institutional ethics committee approval.

The study period was from January 2016to May 2019. Details such as clinical history and the results of investigation were collected from the coagulation profile records maintained in the Clinical Pathology laboratory. If the patient was an in-patient, further details were retrieved from the files in the Medical Records Department (MRD).

EXCLUSION CRITERIA

- Quantitative platelet disorders
- Acquired disorders of platelet function
- Acquired disorders of secondary hemostasis(DIC , liver disease, Vitamin K deficiency)

Whenever a patient presented to the Clinical Pathology &haematology Laboratory division of PSG Hospitals Diagnostic Services for an investigation for abnormal bleeding, as a first step, a detailed history is

obtained. This includes age, sex, presenting complaints,past history of bleeding,type and nature of the bleeding,whether the bleeding was spontaneous or induced,age of onset of significant bleeding symptoms, family history and history of consanguinity.After obtaining clinical details, the patient undergoes a series of **first line screening investigations**. These are:

S.No	Name of test	Test method	Lab's reference range
1	Complete Blood Count	Automated cell counter (VCS technology)	Hb – 13-17g/dl (adult males); 12-16 g/dl(adult females) TLC – 4000-11000 / cu.mm Platelet count – 1.5 -4 lakhs / cu. mm
2	Peripheral smear	Leishman stain, Microscopy	NA
3	Bleeding Time	Ivy method	1 – 6 minutes
4	Clotting time	Lee & White Method	5 – 10 minutes
5	Clot retraction time	Direct observation	30sec – 4 hours
6	Prothrombin time	Photo optical detection of clot	9 – 12s
7	Activated partial Thromboplastin time	Photo optical detection of clot	25 -34s
8	Thrombin time	Photo optical detection of clot	14 -21s
9	Fibrinogen	Clauss method	150 – 400 mg/dl
10	Factor XIII activity	5 molar Urea solubility	Clot should be insoluble after 24 hours.

The results of the first line screening tests would indicate if the cause was platelet disorder or coagulation disorder or both. In the case of the latter two, the following **second line investigations** are done.

- Mixing studies
- Factor Assays
- von Willebrand factor Antigen assay

  If the cause was platelet disorder, platelet function study would be done.

Samples for complete blood count were collected in tube containing K_2EDTA (Ethylene diamine tetra acetic acid) anticoagulant. The sample is barcoded and loaded on to Beckman Coulter (LH780 analyzer). The Beckman Coulter analyzer works on Electrical impedance principle. From Beckman coulter the results are sent to Laboratory Information System (LIS) and then on to the Hospital information system (HIS).

BLEEDING TIME

Bleeding time is used to assess the platelet function and vascular function, more for the former. It is a measure of Primary Hemostasis. The Ivy method is used in our laboratory.

Blood pressure cuff is applied to patient's arm and is inflated to 40 mmHg then the volar aspect of forearm is cleaned with spirit and a clean incision is made with a sterile blade. Simultaneously the stop watch is on, then the filter paper is applied gently over the wound for every 30 seconds and it will

be repeated for every 30 seconds until the bleeding starts completely.After the bleeding stops, the blood pressure cuff is deflated and the time period from the incision until the bleeding has stopped completely is recorded.

Normal bleeding time is 1 to 6 minutes

CLOTTING TIME: It is the time required for the blood to clot.

Test tube method: 2 ml of venous blood is taken in a glass test tube and the clotting time is noted. Prolonged Clotting time indicates abnormalities in secondary phase of hemostasis.

Normal clotting time is 5 to 10 minutes.

Blood samples for coagulation tests such as PT, APTT are collected from venepuncture in blue topped bottles which contain 3.2% sodium citrate as an anticoagulant. For routine coagulation tests Platelet poor plasma (PPP) is used.

Clot retraction time: In this test the blood without anticoagulant is allowed to clot and then allow to stand for some time. This is mostly done by observing the test tubes which are used in clotting time. The test tube is kept in a water bath at 37^0C. The clot begins to retract after 30 minutes and is well appreciated after 2 – 3 hours, though it may not be complete till 12 - 24 hours.

Preparation of PPP: For preparation of platelet poor plasma the blood samples in 3.2% trisodium citrate test tubes are centrifuged at 3500 rpm for

15 min. After centrifugation, plasma is separated ensuring that no platelets are included. In PPP, the platelet count should be <10,000/cu mm.

The sample is bar coded and loaded on to automated coagulation analyzer SYSMEX CS 2400.The coagulation analyzer works on photo optical detection of clot mechanism.

PROTHROMBIN TIME

Principle: The time taken for a sample of citrated plasma to clot after addition of tissue Thromboplastin and calcium chloride. Clot is measured optically by light Scatter and percentage detection method [(42)] .

Prothrombin test measures the factors involved in Extrinsic and Common pathways such as Factor I, II, V, VII and X and the results are reported as the Prothrombin time in seconds with the reference range and the INR.

Required equipment and reagents include

1. Dade Innovin prepared from purified recombinant tissue factor combined with Thromboplastin, calcium, buffers and stabilizers.
2. Control plasma N
3. Control plasma citrol
4. Equipment Required: CS-2400

Reagents are prepared by reconstituting one vial of lyophilized Dade Innovin Reagent with 4 ml. of distilled water and the contents are mixed thoroughly.

Calculation of INR**:**Patient Test value/ Geometric mean^{ISI}

The ISI value and geometric mean of each reagent lot is entered in the CS-2400, CS-1600 analyzers and the calculated INR is obtained.

The result is reported as the mean of the readings in seconds or as a ratio `R' or as international Normalized Ratio (INR). To obtain the ratio the reaction time of the sample is divided by the control.

The value of control, i.e. logarithmic mean normal PT (LMNPT) is obtained by testing the citrate plasma drawn from 20 normal men and women (non pregnant and are not on oral anticoagulant therapy) and the logarithmic mean normal PT is calculated from those 20 values. Before the 20 normal citrate plasma samples are tested for PT, all of them are checked for their platelet counts which should be less than 10,000/uL.

$$R = \frac{\text{Reaction time of test plasma in seconds}}{\text{Control (seconds)}}$$

$$INR = R^{ISI}$$

Therapeutic ranges for INR vary depending on the indication for oral anticoagulant therapy

ACTIVATED PARTIAL THROMBOPLASTN TIME

Principle and method

The time taken for citrated plasma to clot with addition of an activator, phospholipid and $Cacl_2$.It measures factors of Intrinsic and Common pathways such as FactorI, II, V, VIII, IX, X, XI and XII.APTT is also used as a monitoring test for haemorrhagic and thrombotic disorder.

Required equipment and reagents include:

1. Dade Actin-FSL reagent that contains purified soya and rabbit brain phosphatides in ellagic acid, added buffer, stabilizers and preservatives.
2. $CaCl_2$ solution
3. Control plasma N
4. Control plasma citrol
5. CA- coagulation analyzer.
6. Equipment Required: CS-2400

Reagent is prepared by reconstituting one vial of lyophilized Dade Actin-FSL reagent is 2 ml. of distilled water and the contents are mixed thoroughly by inversion.

Interference: APTT results may be affected by drugs like Heparin, Warfarin, Lupus anticoagulant

Resultsare reported as the APTT in seconds with the reference range.

THROMBIN TIME

Principle:Thrombin converts fibrinogen in plasma to fibrin results in clot and time to form is clot is measured.

Reagents required are

- Test thrombin reagent
- Buffer solution

Reference interval is 14 to 21 seconds.

FIBRINOGEN ASSAY: It is a functional assay based upon the time for fibrin clot formation and is done by Clauss method.

Principle: The addition of thrombin coagulates fresh citrated plasma. The clotting time is proportional to the fibrinogen concentration and it is measured by functional clotting assay.

Reagents required are

1. Thrombin reagent – lyophilized preparation from bovine source – 50 NIH units per vial.
2. Fibrinogen calibrator
3. Owren's buffer.

CLOT SOLUBILITY TEST

Principle:Fibrin clots formed in the presence of thrombin & factor XIII are stable for minimum 1 hour in 5M urea and the clots formed in the absence of factor XIII dissolve rapidly.

Reagents required are: 5 M Urea, thrombin reagent, $Cacl_2$,Platelet poor plasma of patient, normal pooled plasma.

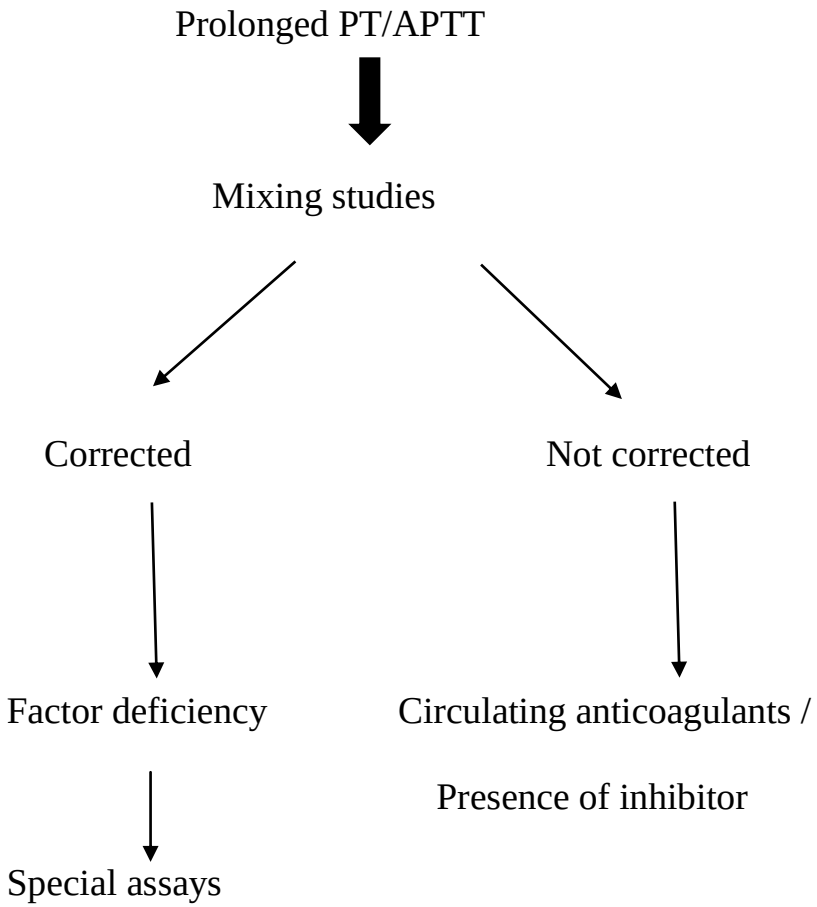
Interpretation

The control clot, if normal shows no sign of dissolving after 24 hours. If the clot dissolvesthat indicates absence of factor XIII. If the clot does not dissolve sufficient factor XIII is present in plasma

MIXING STUDIES

Mixing studies are performedwhen there is prolongation of PT, APTT.

Principle: Mixing studies used to determine whether the prolongation of PT, APTT is due to factor deficiency or presence of inhibitor.Correction of the abnormality by adding an agent indicates that the reagent contains the substance which is deficient in the test sample.



Reagents needed for mixing studies are

- Patient's platelet poor plasma
- Control platelet poor plasma
- Aged serum
- Adsorbed plasma

Aged serum

Aged serum lack the substances which have been consumed in the clotting and lost their activity of Factors I, II, V, VIII. Aged serum contains factors IX,X,XIand XII. As the fresh serum still contains some active thrombin, the serum should be incubated for few hours or overnight now this is called aged serum which contains no thrombin activity.

Adsorbed plasma

Adsorbed plasma is prepared using aluminum hydroxide. By adding 0.4 ml of aluminum hydroxide gel to 2 ml of plasma, mixed for 5 minutes and then centrifuged for 20 minutes and the supernatant is decanted to other tube and used. Because of the lability of Factor V & VII, the adsorbed plasma should prepared fresh.

The adsorbed plasma removes factor II, VII, IX and X and contains factor I, V, VII, XI, XII

Method:

If there is prolongation of PT, APTT then mixing studies are performed with 50:50 mixture of patient's pooled plasma and control pooled plasma. If PT, APTT gets corrected with half of normal pooled plasma and half of patient's control plasma that indicates Factor deficiency.

Equal volumes of patient's plasma and normal control plasma is mixed and the test is repeated. If the presence of half volume of normal plasma corrects the defect, that indicates there is deficiency of one or more factors in the plasma. No correction with half volume of normal plasma indicates that the prolongation of clotting time in patient's plasma is due to circulating anticoagulant or antibody to the factors present in plasma.

Equal volumes of patient's plasma and adsorbed normal plasma is mixed andthe test is repeated. If the presence of half volume adsorbed normal plasma corrects the defect, indicates that the deficient factors should be one of those factors present in adsorbed plasma (i.e.) Factors I, V, VIII, XI or XII.

Then the equal volumes of patient's plasma is mixed with aged normalserum. If the presence of half volume aged normal serum corrects the defect, indicates that the deficient factors would be one of those factors in aged serum.

Interpretation of mixing studies

PT	APTT	Correction with aged serum		Correction with adsorbed plasma		Possible factor deficiency
		PT	APTT	PT	APTT	
Normal	Abnormal	-	Corrected	-	Corrected	XI/XII deficiency
Normal	Abnormal	-	Corrected	-	Not corrected	IX deficiency
Normal	Abnormal	-	Not corrected	-	Corrected	VIII deficiency
Abnormal	Normal	Corrected	-	Not corrected	-	VII deficiency
Abnormal	Abnormal	Not corrected	Not corrected	Not corrected	Not corrected	II deficiency
Abnormal	Abnormal	Corrected	Corrected	Not corrected	Not corrected	X deficiency
Abnormal	Abnormal	Not corrected	Not corrected	Corrected	Corrected	V deficiency

Factor assays

Factor VIII assay

Principle and method**:**Plasma deficient in any of the factors of the intrinsic pathway will result in a prolonged activated partial thromboplastin time

(APTT). Coagulation factor deficient plasma can be used to confirm a factor deficiency in general and to identify and quantify coagulation factor deficiency in a patient's plasma. A mixture of the respective factor deficient plasma and the patient's plasma is tested in theAPTT assay and the result is interpreted using a reference curve obtained with dilutions of Standard Human Plasma. If the patient's plasma is deficient in a particular factor it will not be able to compensate for the absence of the factor in the corresponding factor deficient plasma. This will result in a prolonged APTT

Patient preparation:Heparin therapy should be avoided 3 days prior to the test.

Required equipment and reagents are

1. Lyophilized Factor VIII deficient plasma
2. Dade Actin-FS reagent that contains purified soy phosphatides and rabbit brain phosphatides in ellagic acid, added buffer, stabilisers and preservatives.
3. $CaCl_2$ solution
4. Control plasma N
5. Control plasma P

Results:

The results are reported as percentages of the normal.

The biological reference range for the factor VIII is 70-150% of the normal.

Alert/critical value for Factor VIII levelis<1%.

FACTOR IX ASSAY

Required equipment and reagents are

1. Lyophilized Factor IX deficient plasma
2. Dade Actin-FS reagent that contains purified soy phosphatides and rabbit brain phosphatides in ellagic acid, added buffer, stabilizers and preservatives.

3.$CaCl_2$solution

4.Control plasma N

5.Control plasma P

6.Equipment Required: Coagulation analyzer CS-2400

Results:

The results are reported as percentages of the normal.

The biological reference range for the factor IX is 70-120% of the normal

Alert / critical value for Factor IX level is <1%

FACTOR XI ASSAY

Reagents required are

1. Lyophilized Factor XI deficient plasma
2. Dade Actin-FS reagent that contains purified soy phosphatides and rabbit brain phosphatides in ellagic acid, added buffer, stabilizers and preservatives.
3. $CaCl_2$ solution

4. Control plasma
5. 5.Control plasma P

Results:

The results are reported as percentages of the normal.

The biological reference range for the factor XI is 70-120% of the normal

Alert /critical value for Factor XI level is<1%

Factor XII deficiency

Reagents include

Lyophilized Factor XII deficient plasma. Others are same as Factor VII,IX and XII.

Results:

The results are reported as percentages of the normal.

The biological reference range for the factor XIIis 70-120% of the normal

Alert /critical value for Factor XII level is <1%

VWF ANTIGEN ASSAY

Principle and method:

Small polystyrene particles to which specific antibodies have been attached by covalent bonding are aggregated when mixing with samples containing von Willebrand antigen. This aggregation is detected turbidemetrically via the increase in turbidity, which is proportional to the antigen level present in the test sample.

Required equipment and reagents are

1. vWF Reagent
2. vWF Diluent
3. vWF Buffer
4. Standard Human Plasma (SHP) for calibration.
5. OVB
6. Control Plasma N
7. Control Plasma P
8. Equipment Required: Coagulation analyzer CS-2400

Reagents are prepared bymixing 4 ml. of the given vWF Diluent with 2 ml of vWF reagent and the OVB gets automatically diluted within the instrument.

Results:

- The results are reported as percentages of the normal.
- The biological reference range for the vWF antigen is 50-160% of the normal.

Alert/critical values for vWF is<1% of normal.

During the study period i.e. January 2016 to May 2019, the Clinical Pathology and Hematology Laboratory received 10,69,047 number of samples. Of these, 7,24,032 were Hematology (68%) samples and 3,45,015 were Clinical Pathology (32%) samples.

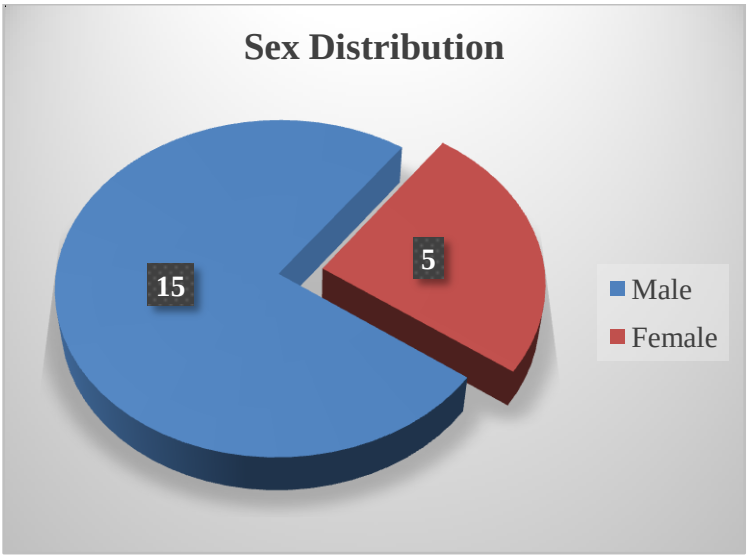
The Hematology testing Laboratory has a coagulation testing division. Of the 7,24,032 samples received for hematology, samples for coagulation testing accounting for 1,21,431(16.8%). The commonly performed coagulation tests are PT, APTT and Fibrinogen followed by D - dimer assay and Thrombin time assay. One unique and exhaustive test done in the coagulation testing division is "Investigation of Abnormal Bleeding". We received 55 numbers of patients seeking this investigation. This accounts for 0.05% of Coagulation tests and 0.007% of Hematology tests.

Of the 55 patients who were investigated for either a clinically obvious abnormal bleeding or history of abnormal bleeding, 21 patients did not have any hemostatic abnormality. 11patients had acquired bleeding disorders and 20 patients had congenital bleeding disorders as shown in the table below. Three patients had platelet function disorders as proven by platelet function studies.

S NO	Diagnosis Group	No of patients
1	**Inherited bleeding disorders**	**20**
2	Acquired bleeding disorders	11
3	Platelet Function disorders	03
4	No hemostatic abnormalities	21
	Total	**55**

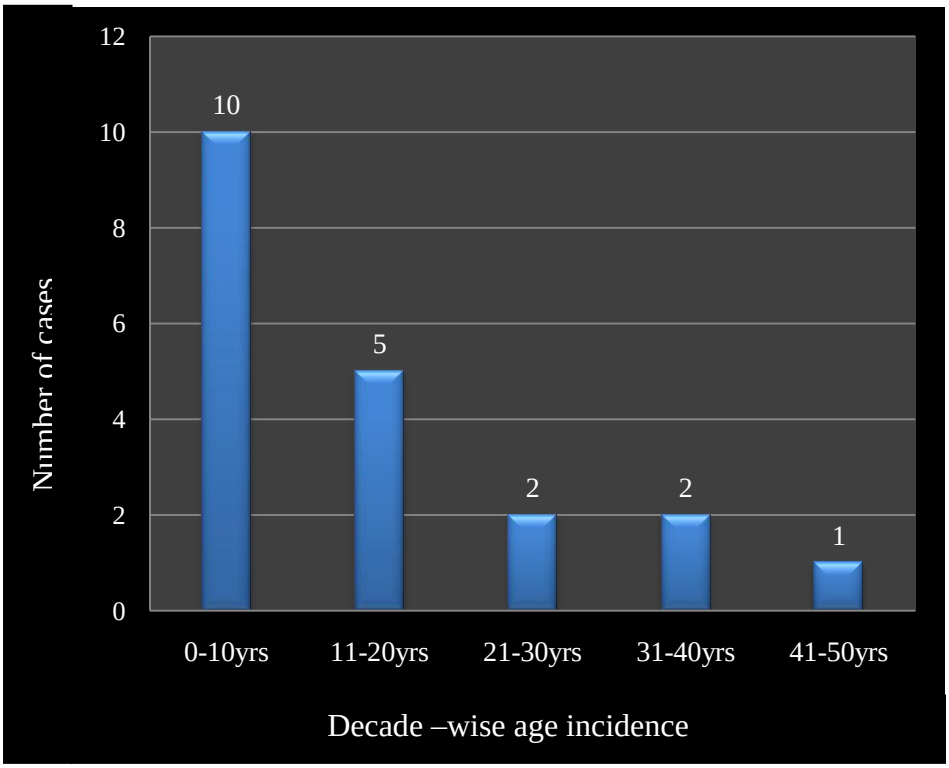
It is these 20 patients with inherited bleeding disorders who comprise the study population.

The diagram below displays the sex distribution in the study population.



It is evident that Males outnumber Females in the ratio of 3:1.

The bar diagram indicates the decade wise incidence of age at diagnosis of the inherited bleeding disorders.



The age range in the study population varied from 6 months to 41 years with a mean age of 14 years and median of 11 years.

The table below displays the age at diagnosis based on sex of the patient.

S No	Decade	No of Male patients	No of female patients
1	1 to 10 years	8	2
2	10 to 20 years	4	1
3	21 to 30 years	2	1
4	31 to 40 years	1	-
5	41 to 50 years		1
		15	5

Most of the male patients (53%) presented in the first decade itself. Females have an almost equal distribution for each of the age decades. The youngest male was aged 5 months and the oldest male was 40 years of age.

The youngest female was aged 6 months and the oldest female was 41 years of age.

The table below displays the various types of inherited bleeding disorders encountered during the study period.

Inherited Bleeding Disorder	No of patients
Von Willebrand Factor deficiency	5
Factor VII deficiency	1
Factor VIII deficiency	8
Factor IX deficiency	2
Factor X deficiency	1
Combined Factor deficiency	3
Total	20

The most common type of inherited bleeding disorders is Haemophilias which account for 50% of cases. Hemophilia A alone accounted for 40% of cases.

VWD is the next most common inherited factor deficiency accounting for 25% of cases.

Factor VII & Factor X deficiency are rare bleeding disorders which accounts for 10% of cases (one of each).

Combined factor deficiencies account for 15% of inherited bleeding disorders.

In all of these three, Factor VIII deficiency was the commonest associate. Hence, overall there are eleven cases of Factor VIII deficiency which accounts for 55% of the study population.

The table below depicts the severity of Factor VIII deficiency patients in the study population.

Severity of Factor VIII deficiency	No of cases
Mild (6 -30%)	5
Moderate (1-5%)	1
Severe (<1%)	5
Total	11

Of the mild factor deficiencies of factor VIII, the mean Factor VIII value was 24% with values ranging from 8.1% to 37.6%. One patient had moderate deficiency of factor VIII with a Factor VIII activity of 1.3%.

Two patients had isolated mild Factor IX deficiency (2.6% and 13.5%) and another patient was part of a combined deficiency with a factory activity of 2.6%. The mean factor IX activity was 6.2%.

Five patients presented with isolated VWF deficiency while one patient had a combined deficiency of VWF and Factor VIII. The values of VWF ranged from <1% to 12.9%. 50% of the patients with VWD had severe deficiency while, of the remainder, one patient had moderate deficiency and another two patients had mild deficiency. Four of the six VWD patients (66.6 %) diagnosed to have type 3 VWD. Two patients had type I VWD. This information is summarized in the table below.

SNo	VWF activity	Severity of disease	Type of VWD
1	3.3	Moderate	III
2	11.6	Mild	I
3	0	Severe	III
4	0	Severe	III
5	<1	Severe	III
6	12.9	Mild	I

In the study population, 8 patients had bleeding on challenge and 12 patients had presented with spontaneous bleeding as shown in the table below.

Type of bleeding	Number of cases
Bleeding on challenge	08
Spontaneous bleeding	12

Of the 8 patients who bled on challenge, seven had mild deficiency and one had moderate deficiency of coagulation factors.

Of the 12 patients who had presented with spontaneous bleeding, 10 had severe factor deficiencies. These patients include all the 5 patients with von Willebrand Disease, 1 with multiple factor deficiencies and 5 with severe factor deficiencies. Four of the patients with severe factor deficiencies had hemophilia A while one of them had a factor X deficiency. These details are summarized in the table below.

Severity of factor deficiency	Abnormal bleeding on challenge	Spontaneous bleeding
Mild	7	1
Moderate	1	1
Severe	0	10
Total	8	12

60% of the patients in the study (12 cases) presented with muco-cutaneous bleeding, while 8 cases (40%) presented with major bleeding such as Haemarthrosis (2 cases), Haematomas (4 cases), severe menorrhagia (1 case and haemorrhagic pericardial effusion (1 case).

The table below gives the distribution of the clinical bleeding encountered in the study population.

Type of bleeding	Clinical presentation	No of cases	Total
Muco-cutaneous bleeding	Ecchymosis	5	12
	Gum bleeding	2	
	Epistaxis	2	
	Easy bruising	3	
Major Bleeding	Haemarthrosis	2	8
	Haematomas	4	
	Severe menorrhagia	1	
	Haemorrhagic pericardial effusion	1	

PSG Hospitals Diagnostic Services has four Diagnostic Service Laboratories namely Clinical Biochemistry, Clinical Microbiology, Pathology and Molecular Genetics. The Pathology discipline has Clinical Pathology, Hematology, Immunohaematology, Histopathology and Cytopathology divisions. All the service laboratories are accredited by National Accreditation Board for Laboratories.

The Clinical Pathology and Hematology Laboratory is characterized as a large size laboratory receiving between 600 and 800 patient samples every day. All the samples are collected either in the central collection laboratory (for outpatients) or in any of the wards of the hospital (inpatients). Until 2014, the Clinical Pathology and Hematology Laboratory was equipped with a fully automated coagulation analyzer called STA Compact Max of Diagnostica Stago, France.

In mid 2015, the laboratory acquired the first installation in India of a high end coagulation analyzer called CS -2400 of Sysmex, Japan. This machine is unique because it incorporates platelet function tests also. All the coagulation tests for bleeding disorders and thrombophilia can be performed on this machine because this machine has all the three principles such as photo optical detection of clot, immunoassay and chromogenic assays methods incorporated. The machine has an on board Peltier based cooling

system, to store all the different reagents that have been prepared either by reconstitution or after dilution or as such, based on manufacturer's instructions. The equipment is extremely user friendly and has bidirectional interfacing facility which can understand the tests need to be done and performs it with appropriate in-built protocols using the correct reagents. This has drastically brought down the occurrence of analytical errors.

On the CS -2400, all the factor assays have been standardized either as one-stage PT assay or one-stage APTT based assay. The factor assays performed are assays of Factor II, V, VII, VIII, IX and X.

The laboratory has a unique test called “Investigation of a bleeding patient”. For this test alone, the patients report to the laboratory directly. Soon after the registration formalities, postgraduate residents obtain a detailed clinical history based on a standardized template. They also attempt to draw a pedigree chart in relevant cases. As detailed in the materials and methods, a battery of tests is done after the blood drawal while the patient is in the laboratory itself.

The results of the first line screening tests are interpreted by the consultations to identify whether a bleeding disorder exists or not. In all suspected bleeding disorders an attempt is made to identify if it was a disorder of Primary Hemostasis or Secondary hemostasis or both.

In Patients with Primary Hemostasis, if there is no significant thrombocytopenia, Platelet Function Tests are done to investigate for a probable Thrombasthenia. If secondary hemostatic disorders are suspected, mixing studies using normal plasma, adsorbed plasma and aged serum are done to identify which specific factor(s) needs to be assayed. If both disorders are suspected all the relevant tests as described above are done.

The advent of the CS -2400 helped us to make a final diagnosis of the cause of abnormal bleeding. The disorders could be considered congenital or acquired based on the clinical presentations.

Of the 55 patients who were investigated for abnormal bleeding, 36.4% (20 cases) had an inherited bleeding disorder, while 5.5% had a platelet function disorder. This contrasts with the observations made by Gupta M, Bhattarcharya M, Choudary VP and Saxena R where 60.6% had inherited bleeding disorders [(31)]. This is probably because AIIMS is the largest reference centre in India and all the cases of abnormal bleeding from various parts of the country are referred there. Hence this may not be representative for the region.

While eleven cases (20%) in our study were diagnosed as acquired bleeding disorders, Bhushan Asthana, Prashant Sharma, Ravirajan Ranjan et al from AIIMS observed that 77.4% had an acquired bleeding disorders [(33)]. Ideally, acquired bleeding disorders do not required this work up if on

clinical history, a cause for the bleeding disorder could be identified. This exhaustive investigation protocol must only be carried out if there are no obvious acquired causes for the abnormal bleeding.

75% of the patients in our study population with inherited bleeding disorders were males. This is similar to the observations in most of the study as the most common congenital bleeding disorders was the Hemophilia A & B, X linked recessive disorders seen most commonly in males.

The age range for our population varies from 6 months to 40 years. In the study from AIIMS Delhi published in 2005, the age range was from birth to 35 years. However, Peyvandi F, Palla R, Menegatti in 2012 found a similar age range as ours ranging from 7 months to 95 years [(37)].

Most of the male patients presented in the first decade of life followed by the second decade with a median age at diagnosis of 11 years and the mean age of 14 years. In Peyvandi F, Palla R, Menegetti et al study the mean age was 31 years [(37)].

50% of the inherited bleeding disorders were the Hemophilias of which Hemophilia A comprises 80% (8 cases) and Hemophilia B comprises 20% (2 cases). Studies published in NEJM in 2001, from AIIMS in 2005, from AIIMS in 2008 and by researchers from Italy and Iran all showed similar trends [(30, 31, 32, and 35)]. However, In Iraq, 90% of the cases were Hemophilia

A or VWD, owing to the very high occurrence of consanguineous marriage [35].

VWF deficiency comprises 25% of the study population which is similar to most studies in the literature.

Rare bleeding disorders account for 10% of the study population with one case each of factor VII, X deficiency. Acharya SS et al observed that in North America, Factor VII deficiency was the most common rare bleeding disorder [29]. However, the study from AIIMS published in 2012 shows that 43% of rare bleeding disorders were due to Factor X deficiency followed by deficiencies of factor XIII and VII [36]. In the same year Peyvandi F, Palla R, Menegetti et al observed that factor VIII, XI were the most common causes for Rare bleeding disorders in Europe[37]. Thus it is clear that the incidence of rare bleeding disorders, is different between India and the rest of the world. Even in the most recent study published in 2019 by Menegetta M, Palla R the most common causes of rare bleeding disorder continue to be factor VII and XI respectively[41].

There was an almost equal distribution of severe and mild deficiencies of factor VIII in our study. We did not observe a correlation of severity of clinical bleeding and factor assays for the most common deficiencies such as factor VIII, IX and VWF. This is similar to the observations from the researches from CMC Vellore [38].

67% of vWD were of type III (4 out of 6 cases). Of these, three had severe deficiencies of vWF activity. The findings of Ahmed F Kannan, Ranjan et al contrasts this as they found that 54% cases of vWD in the study were of type 2 [(32)].

40% of the patients in the study population had grade 3 bleeding, while 60% had grade 1 or 2 bleeding. The severe bleeding included those with intramuscular hematomas, Haemarthrosis and Hemorrhagic Pericardial effusion. In Peyvandi F, Palla R study only 12.5% had grade 3 bleeding. This is because their study was mainly restricted to rare bleeding disorders.[(37)]

SUMMARY

1. 16.8% of samples received by Hematology Laboratory were for coagulation testing of which, 0.05% were for "Investigation of Abnormal Bleeding."
2. Of the 55 patients who were investigated for abnormal bleeding, 20 patients (36.4%) were diagnosed to have an inherited bleeding disorder (IBD).
3. 75% of the patients with inherited bleeding disorders were males.
4. The age range of those with inherited bleeding disorders was from 6 months to 41 years. Most patients were in the first and second decades.
5. Hemophilia A & B account for 50% of the inherited bleeding disorders followed by vWD.
6. Rare inherited bleeding disorders include deficiencies of factor VII & X.
7. 3 patients had combined factor deficiencies.
8. 4 out of 6 vWD patients were of type III.
9. 60 % had mucocutaneous bleeding while 40 % had major bleeding (grade III)
10. There was no correlation between severity of factor VIII deficiency and clinical bleeding.

CONCLUSION

Fully automated coagulation analyzers with appropriate reagents, internal quality control and proficiency testing activities are a boon to identify inherited bleeding disorders. The Haemophilias and vWD continue to be the most common causes for congenital disorders of hemostasis. More number of cases needs to be studied to analyze the clinico-pathological profile of rare bleeding disorders. Extensive work up is required to identify patients with multiple factor deficiencies.

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MASTER CHART

S NO	Age	Sex	Inheritance	Spontaneous/challenge	Current history	CBC(Platelet count /cumm	Bleeding time (BT)	Clotting time (CT)	Prothrombin time (PT)	Activated partial thromboplastin time(APTT)	Mixing studies	Factor assays	Diagnosis
1	20	M	AR	On challenge	Easy briuisability	Normal	N	N	↑	N	-	VII - decreased	Mild factor VII Deficiency
2	13	M	XLR	On challenge	Hematomas in lower extremities	Normal	N	N	N	↑	APTT - corrected with with aged serum	IX - decreased(13.5%)	Hemophilia B
3	26	M	XLR	On challenge	Hemarthrosis & easy bruising	Increased	N	N	N	↑	APTT - corrected with with aged serum	IX - decreased	Hemophilia B
4	7	M	XLR	On challenge	Hemarthrosis & easy bruising	Increased	N	N	N	↑	APTT - partially corrected wth aged serum & adsorbed plasma	VIII - (8.1%)	Mild hemophilia
5	6 months	F	AR	Spontaneous	Epistaxis	Increased	↑	N	N	↑	APTT - corrected with 1/2 patient	VIII - decreased (3.3%)	VWD type III
6	9 months	M	AR	Spontaneous	Hemorrhagic pericardial effusion	Normal	N	N	N	↑	APTT - partially corrected with normal pooled plasma and aged serum ,fully corrected withadsorbed plasma	VIII - decreased(10.5%),IX-decreased (2.6%)	Combined factor VIII and IX deficiency

MASTER CHART

7	20	F	AR	On challenge	easy bruising	Normal	N	N	↑	↑	PT - corrected with normal pooled plasma,APTT-corrected with pooled plasma&aged serum	V - decreased(11.7%),VII-decreased(11.7%),VIII-decreased (15.9%)	Combined factor V,VII,VIII deficiencies
8	5 months	M	XLR	1	Hematomas & ecchymoses	Normal	-	↑	N	2	APTT - corrected with normal pooled plasma &adsorbed plasma	VIII - decreased (<1%)	Hemophilia A
9	10	F	AD	Spontaneous	Ecchymosis	Normal	↑	N	N	N	-	VIII - mildly reduced (43.3%), VWF antigen assay - moderately reduced(11.6%)	VWD type 1
10	5	M	XLR	Spontaneous	Painful swelling & discoloration over lower back &abdomen	Normal	↑	N	N	↑	APTT - corrected with normal pooled plasma,partially corrected with adsorbed plasma,not corrected with aged serum	VIII - markedly reduced (<1%)	Severe Hemophilia A

MASTER CHART

11	13	M	XLR	1	Painful swelling with bluish black discoloration over right ankle	Normal	N	b	N	↑	APTT - corrected with normal pooled plasma,partially corrected with adsorbed plasma,not corrected with aged serum	VIII - markedly reduced (<1%)	Severe Hemophilia A
12	37	F	AD	Spontaneous	Severe menorrhagia	Normal	N	N	N	↑	-	VIII - decreased(8%),VWF antigen (0%)	VWD
13	41	F	AR	Spontaneous	Gum bleed	Normal	N	N	N	↑	Corrected with normal pooled plasma &aged serum	VIII- reduced(6.6%),VWF antigen - reduced (0%)	VWD type III
14	1	M	AR	Spontaneous	Ecchymosis	Normal	-	↑	↑	↑	PT,APTT - corrected with aged serum and adsorbed plasma	Factor X - decreased (0%)	Severe factor X deficiency
15	8	M	XLR	On challenge	Easy bruising	Normal	N	N	N	↑	APTT - corrected with normal pooled plasma	VIII - decreased (1.3%)	Hemophilia A
16	10 months	M	XLR	Spontaneous	Ecchymosis	Normal	N	↑	N	↑	APTT- corrected with normal pooled plasma	VIII - (<1%),VWF antigen - decreased(12.9%)	Severe Hemophilia A with VWD

MASTER CHART

17	1	M	XLR	Spontaneous	Ecchymosis	Normal	-	b	N	↑	APTT - corrected with normal pooled plasma	VIII - decreased (<1%)	Severe Hemophilia A
18	24	M	AR	Spontaneous	Gum bledding	Normal	↑	a	N	↑	APTT - corrected with normal pooled plasma	VIII - decreased(16.6%),VWF antigen assay - reduced(<1%)	VWD type III
19	12	M	XLR	On challenge	Hematomas	Increased	N	-	N	↑	APTT - corrected with normal pooled plasma	VIII - decreased (47.7%)	Hemophilia A
20	40	M	XLR	On challenge	Hematomas	Increased	N	-	N	↑	APTT - corrected with normal pooled plasma	VIII - decreased (37.7%)	Hemophilia A
				1- Spontaneous, 2-Induced		1-Normal (150 to 350 x 10^3 / µl, 2-increased	N-Normal (1 to 7 min),↑- Prolonged	N-Normal (8 min 56 sec),↑- Prolonged	N-Normal(9 to 12 sec),↑- Prolonged	1 - Normal (25 to 34 sec), 2 - Prolonged			VWD - von willibrand disease