STUDY OF VARIABLE CLINICAL PRESENTATIONS IN PATIENTS WITH SOME INHERETED THROMBOPHILIA

Thesis

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Master Degree in **Internal Medicine**

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INTRODUCTION

The human hemostatic system consists of multiple independent, yet integrally related, cellular and protein components that function to maintain blood fluidity under normal conditions and promote localized, temporary thrombus (hemostatic thrombus) formation at sites of vascular injury. A normal hemostatic system is the human physiologic defense against exsanguination. An abnormal hemostatic system can result in pathologic bleeding, vascular thrombosis, or both ***(Schmidt, 2007).***

The hemostatic system is comprised of six major components:

1. Platelets.
2. Procoagulant plasma protein "factors" .
3. Natural anticoagulant proteins.
4. Vascular endothelium.
5. Fibrinolytic proteins.
6. Antifibrinolytic proteins.

Each of these six hemostatic components must be present in fully functional form, in adequate quantity, and at the proper location to prevent excessive blood loss after vascular trauma and, at the same time, to prevent pathologic thrombosis. The hemostatic system is highly regulated and maintains a

delicate balance between a prohemorrhagic state and a prothrombotic state.

Any significant acquired or congenital imbalance in the hemostatic “scales” can lead to a pathologic outcome.

Normal hemostasis in response to vascular injury can be divided into two major processes of equal importance known as primary and secondary hemostasis. Primary hemostasis comprises the reactions needed to form a platelet plug at a site of vascular damage, whereas secondary hemostasis comprises a series of reactions ***(***coagulation cascade) needed to generate cross-linked fibrin required to stabilize the platelet plug and form a durable thrombus. Natural anticoagulants [antithrombin ***(***AT) and activated protein C] function to confine thrombus formation to the sites of vascular injury and limit thrombus size to prevent vessel occlusion and flow interruption in the affected vessel. The activity of AT is greatly enhanced by endothelial cell heparan sulfate and pharmacologic heparins ***(Brewer, 2006).***

The function of activated protein C ***(***APC) is enhanced by its cofactor, protein S.

Physiologic fibrinolysis is initiated by endothelial cell–derived tissue-type plasminogen activator ***(***t-PA), which converts plasminogen to plasmin. Plasmin can degrade cross-linked fibrin, limit thrombus size, and help dissolve a thrombus once the vascular injury has been repaired. The fibrinolytic

system is regulated and localized by antiplasmin and plasminogen activator inhibitor ***(***PAI**) *(Broderick, 2002).***

Specific alterations in the quantitative and qualitative status of any hemostatic cellular or protein element can lead to pathologic thrombosis. A marked increase in the platelet count ***(***thrombocytosis) and accentuated platelet aggregation ["sticky platelet syndrome" ***(***SPS)] are associated with thromboembolic events. Elevated levels of procoagulant factors such as factor VIII, fibrinogen, factor IX, factor XI, and factor VII, as well as factor V resistance to inactivation by activated protein C, are recognized risk factors for vascular disease and thrombosis. Deficiency of a natural anticoagulant protein such as protein C, protein S, and AT is associated with venous thromboembolic disease.

Deficiency of a fibrinolytic cascade component, such as t-PA or plasminogen, and excess plasma levels of the fibrinolytic inhibitor PAI-1 have been linked to hypercoagulability and thrombosis. Deficient endothelial cell production of thrombomodulin or release of t-PA may be associated with a thrombotic tendency. It is the net balance between the participating and, at times, opposing groups of proteins and not the level of any individual factor that is most critical to hemostatic regulation ***(Deitcher et al., 2001).***

AIM OF THE WORK

The aim of this work is to study different clinical presentation in patients with thromophilia with inherited hypercoagulable conditions, with its relation to certain genes such as mutation in factor V-leiden, prothrombin 20201A and MTHFR detected by PCR among Egyptian patients.

PHYSIOLOGY OF HEMOSTASIS

1- Platelet Activation:

Damage to blood vessel walls exposes subendothelium proteins, most notably von Willebrand factor (vWF), present under the endothelium. vWF is a protein secreted by healthy endothelium, forming a layer between the endothelium and underlying basement membrane. When the endothelium is damaged, the normally-isolated, underlying vWF is exposed to blood and recruits Factor VIII, collagen, and other clotting factors. Circulating platelets bind to collagen with surface collagen-specific glycoprotein Ia/IIa receptors. This adhesion is strengthened further by additional circulating proteins vWF, which forms additional links between the platelets glycoprotein Ib/IX/V and the collagen fibrils. These adhesions activate the platelets ***(Brewer, 2006).***

Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet-activating factor (PAF), vWF, platelet factor 4, and thromboxane A_2 (TXA_2), which, in turn, activate additional platelets. The granules' contents activate a G_q-linked protein receptor cascade, resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C, which, in turn, activates phospholipase A_2 (PLA_2). PLA_2 then modifies the integrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelets

change shape from spherical to stellate, and the fibrinogen cross-links with glycoprotein IIb/IIIa aid in aggregation of adjacent platelets ***(Giangrande, 2003).***

2- Coagulation Factors:

The coagulation cascade:

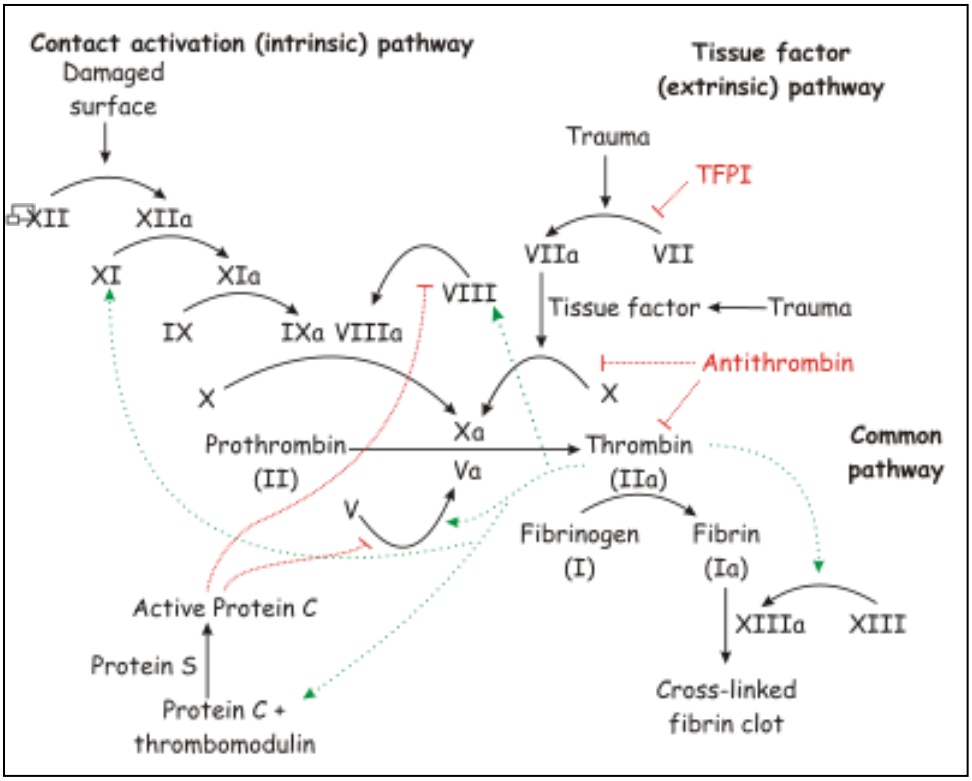

Figure (1): The coagulation cascade ***(Furie, 2005).***

The coagulation cascade of secondary hemostasis has two pathways, the *contact activation pathway* (formerly known as the intrinsic pathway), and the *tissue factor pathway* (formerly known as the extrinsic pathway), which lead to *fibrin*

formation. It was previously thought that the coagulation cascade consisted of two pathways of equal importance joined to a common pathway. It is now known that the primary pathway for the initiation of blood coagulation is the *tissue factor* pathway. The pathways are a series of reactions, in which a zymogen ***(***inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase *a* appended to indicate an active form ***(Furie, 2005).***

The coagulation factors are generally serine proteases (enzymes). There are some exceptions. For example, FVIII and FV are glycoproteins, and Factor XIII is a transglutaminase. Serine proteases act by cleaving other proteins at specific sites. The coagulation factors circulate as inactive zymogens. The coagulation cascade is classically divided into three pathways. The *tissue factor* and *contact activation* pathways both activate the "final common pathway" of factor X, thrombin and fibrin.

Tissue Factor Pathway *(*Extrinsic):

The main role of the tissue factor pathway is to generate a "thrombin burst," a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released instantaneously. FVIIa

circulates in a higher amount than any other activated coagulation factor ***(Schmidt, 2007).***

- Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).
- TF-FVIIa activates FIX and FX.
- FVII is itself activated by thrombin, FXIa, plasmin, FXII and FXa.
- The activation of FXa by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).
- FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.
- Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which activates FXI, which, in turn, activates FIX), and activates and releases FVIII from being bound to vWF.
- FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes) ***(Brewer, 2006).***

Contact Activation Pathway *(*Intrinsic):

The contact activation pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that patients with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder ***(Shapiro, 2003).***

Final Common Pathway:

Thrombin has a large array of functions. Its primary role is the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.

Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down-regulated by the anticoagulant pathways ***(Giangrande, 2003).***

Cofactors:

Various substances are required for the proper functioning of the coagulation cascade:

Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets, as well as procoagulant microparticles or microvesicles shed from them. Calcium is also required at other points in the coagulation cascade ***(Furie, 2005).***

- Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X, as well as Protein S, Protein C and Protein Z. In adding the gamma-carboxyl group to glutamate residues on the immature clotting factors Vitamin K is itself oxidized. Another enzyme, *Vitamin K epoxide reductase*, (VKORC) reduces vitamin K back to its active form. Vitamin K epoxide reductase is pharmaco-logically important as a target for anticoagulant drugs warfarin and related coumarins such as acenocoumarol, phenprocoumon, and dicumarol. These drugs create a deficiency of reduced vitamin K by blocking VKORC, thereby inhibiting maturation of clotting factors. Other deficiencies of vitamin K (e.g., in malabsorption), or disease

(hepatocellular carcinoma) impairs the function of the enzyme and leads to the formation of PIVKAs (proteins formed in vitamin K absence); this causes partial or non-gamma carboxylation, and affects the coagulation factors' ability to bind to expressed phospholipid ***(Schmidt, 2007).***

3-Regulators and Natural Anti-Coagulant:

Five mechanisms keep platelet activation and the coagulation cascade in check. Abnormalities can lead to an increased tendency toward thrombosis:

- Protein C is a major physiological anticoagulant. It is a vitamin K-dependent serine protease enzyme that is activated by thrombin into activated protein C (APC). Protein C is activated in a sequence that starts with Protein C and thrombin binding to a cell surface protein thrombomodulin. Thrombomodulin binds these proteins in such a way that it activates Protein C. The activated form, along with protein S and a phospholipid as cofactors, degrades FVa and FVIIIa. Quantitative or qualitative deficiency of either may lead to thrombophilia (a tendency to develop thrombosis). Impaired action of Protein C (activated Protein C resistance), for example by having the "Leiden" variant of Factor V or high levels of FVIII also may lead to a thrombotic tendency ***(Brewer, 2006).***

- Antithrombin is a serine protease inhibitor (serpin) that degrades the serine proteases: thrombin, FIXa, FXa, FXIa,

and FXIIa. It is constantly active, but its adhesion to these factors is increased by the presence of heparin sulfate (a glycosaminoglycan) or the administration of heparins (different heparinoids increase affinity to FXa, thrombin, or both). Quantitative or qualitative deficiency of antithrombin (inborn or acquired, e.g., in proteinuria) leads to thrombophilia.

- Tissue factor pathway inhibitor (TFPI) limits the action of tissue factor (TF). It also inhibits excessive TF-mediated activation of FIX and FX.

- Plasmin is generated by proteolytic cleavage of plasminogen, a plasma protein synthesized in the liver. This cleavage is catalyzed by tissue plasminogen activator (t-PA), which is synthesized and secreted by endothelium. Plasmin proteolytically cleaves fibrin into fibrin degradation products that inhibit excessive fibrin formation.

- Prostacyclin (PGI_2) is released by endothelium and activates platelet G_s protein-linked receptors. This, in turn, activates adenylyl cyclase, which synthesizes cAMP. cAMP inhibits platelet activation by decreasing cytosolic levels of calcium and, by doing so, inhibits the release of granules that would lead to activation of additional platelets and the coagulation cascade ***(Brwewr, 2006).***

4- Endothelium:

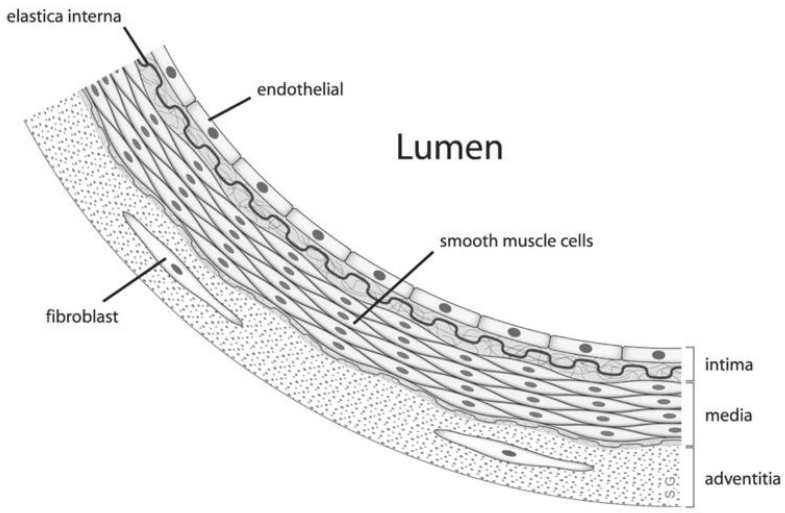

Figure (2): Diagram showing the location of endothelial cells ***(Roberts et al., 2009).***

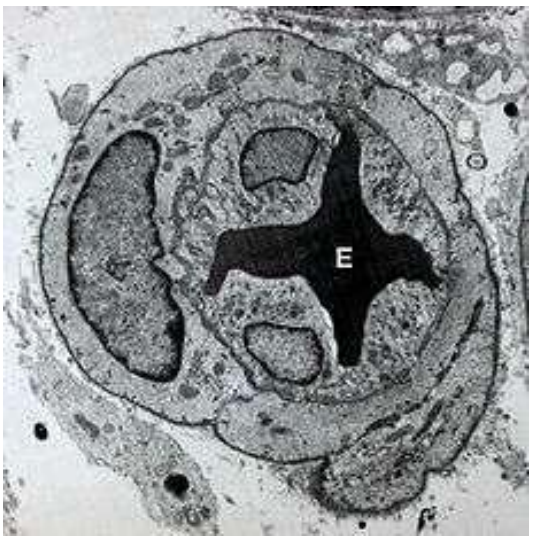

Figure (3): Endothelial cells, which form the tunica intima, encircle an erythrocyte (E) ***(Roberts et al., 2009).***

The **endothelium** is the thin layer of cells that line the interior surface of blood vessels, forming an interface between circulating blood in the lumen and the rest of the vessel wall. Endothelial cells line the entire circulatory system, from the heart to the smallest capillary. These cells reduce turbulence of the flow of blood allowing the fluid to be pumped farther ***(Roberts et al., 2009).***

Blood cell interactions with vessel wall were first documented almost 170 years ago. Modern advances have revealed that leukocyte and platelet interactions with the endothelium are at the nexus of complex, dynamic cellular and molecular networks that when dysregulated may lead to pathological inflammation and thrombosis.

Endothelial tissue is a specialized type of epithelium tissue. More specifically, it is simple squamous epithelium, The endothelium normally provides a non-thrombogenic surface because it contains heparin sulphate which acts as a cofactor for activating antithrombin III, a protease that cleaves several factors in the coagulation cascade ***(Roberts et al., 2009)***.

The foundational model of anatomy makes a distinction between endothelial cells and epithelial cells on the basis of which tissues they develop from and states that the presence of vimentin rather than keratin filaments separate these from epithelial cells ***(Deanfield et al., 2008).***