INTRODUCTION

Thrombin activatable fibrinolysis inhibitor (TAFI, Plasma pro-carboxypeptidase B, carboxypeptidase U) is a single chain glycoprotein zymogen, synthesized in the liver and circulating at a plasma concentration of 4.4–15 μg/ mL **(Davis *et al.*, 2005).** It circulates as a procarboxypeptidase B zymogen, which is converted into an active form, carboxypeptidase U or activated TAFI (TAFIa), during coagulation after thrombin cleavage. Generation of TAFIa is dependent on the quantity of thrombin generated during coagulation and is drastically potentiated by thrombomodulin **(Vague *et al*., 2000).**

Activated TAFI is a member of the family of metallo-carboxypeptidases. These exopeptidases are zinc dependent and cleave carboxy-terminal peptide bond **(Wei *et al.*, 2002).**TAFI is proposed to play a key role in the interaction between procoagulant, anticoagulant and fibrinolytic systems **(Davis *et al.*, 2005).**The direct action of TAFI as an inhibitor of clot lysis involves removal of carboxy-terminal lysyl and arginyl residues from partially degraded fibrin. Consequently, plasminogen binding sites are eliminated and plasminogen activation and fibrinolysis are inhibited **(Monasterioa *et al.*, 2004).**

Endogenous fibrinolysis is a protective mechanism against lasting arterial thrombotic occlusion, which would otherwise lead to permanent tissue damage. Because arterial

thrombogenesis is an active, ongoing, and dynamic process, a healthy endogenous fibrinolytic system can prevent the build-up of thrombus before complete occlusion occurs or break up the occlusive thrombus before lasting tissue damage ensues. Thrombin converts the inactive proenzyme plasminogen to active plasmin. This fibrinolytic system can be inhibited either by antagonizing plasmin through alpha 2-antiplasmin or by specific plasminogen activator inhibitors (PAI) or TAFI **(Gorog, 2010).**

Some studies reported low thrombin activatable fibrinolysis inhibitor antigen (TAFI-Ag) levels in patients with coronary artery disease (CAD) and attributed a cardioprotective role to reduced TAFI levels against major adverse cardiovascular event. Other studies have found CAD associated with raised TAFI levels measured by TAFIa/TAFIai enzyme-linked immunoadsorbent assay (ELISA) but not of the total amount of TAFI to be independently associated with risk of cardiovascular death. There were other studies that were neutral; The TAFI levels were not associated with an increased risk of arterial thrombosis. These conflicting findings of either low or high TAFI levels being associated with cardiovascular risk might be related to the discrepancy between measurements of total TAFI-Ag or TAFI activity, either in all patients or in only those with specific TAFI genotypes **(Gorog, 2010).**

It is still not clear whether total antigen or activity measurements better reflect the physiological situation. Both quantitative and qualitative differences exist between studies measuring either the total antigen levels by the standard commercial ELISA methods and those reporting biological activities. The poor correlation between measurements of TAFI-Ag and activity might be because activated TAFI is quickly inactivated by a rapid spontaneous conversion to the latent form with a half-life of approximately 10 min. Therefore, the very short half-life of TAFIa presents a practical measurement problem, which is difficult to overcome in clinical settings **(Gorog, 2010)**.

AIM OF THE WORK

- Measurement of TAFI level in patients with CAD.
- Determining the correlation between TAFI level and known risk factors of CAD.

Chapter (1)

COAGULATION

An intricate mechanism has evolved in vertebrates to limit blood loss from damaged blood vessels through formation of clot (hemostasis), while maintaining blood in a fluid state where the circulation is intact. This system is required for maintaining the integrity of the circulatory system, and perturbations in the balance between procoagulant and anticoagulant forces can lead to bleeding or thrombotic disorders **(Gailani and Renne, 2007).**

Blood coagulation (the cessation of blood loss from a damaged vessel) is part of an important host defense mechanism. Upon vessel injury, platelets adhere to macromolecules in subendothelial tissues at the site of injury and then aggregate to form the primary hemostatic plug. Platelets stimulate the local activation of plasma coagulation factors, which leads to the generation of a fibrin clot that reinforces the platelet aggregate. Later, as wound healing occurs, the platelet aggregate and fibrin clot are broken down and removed **(Riddel *et al.*, 2007).** In addition, chemokines facilitate the attraction of leucocytes to the area, which prevent infection and assist in wound healing **(Norris, 2003)**.

Hemostasis is regulated by 3 basic components—namely, the vascular wall, platelets, and the coagulation cascade. Normal

hemostasis occurs as the result of a set of regulated processes to accomplish 2 functions; first, it maintains blood in a fluid, clot-free state, and second, it induces a rapid and localized hemostatic plug at the site of vascular injury **(Riddel *et al.*, 2007).**

Coagulation could be initiated via an "intrinsic pathway" so named because all the components were present in blood, or by an "extrinsic pathway" in which the subendothelial cell membrane protein, tissue factor (TF), was required in addition to circulating components. The initiation of either pathway resulted in activation of FX and the eventual generation of a fibrin clot through a common pathway **(Riddel *et al.*, 2007).**

I. Coagulation pathways

A. Intrinsic pathway

The intrinsic pathway consists of a cascade of protease reactions initiated by factors that are present within the blood. When in contact with a negatively charged surface such as glass or the membrane of an activated platelet, a plasma protein called FXII (Hageman factor) becomes FXIIa (the suffix "a" indicates that this is the activated form of FXII). A molecule called high molecular weight kininogen (HMWK), a product of platelets that may in fact be attached to the platelet membrane, helps anchor FXII to the charged surface and thus serves as a cofactor. However, this HMWK-assisted conversion of FXII to FXIIa is limited in speed **(Riddel *et al.*, 2007).**

Once a small amount of FXIIa accumulates, this protease converts prekallikrein to kallikrein, with HMWK as an anchor. In turn, the newly produced kallikrein accelerates the conversion of FXII to FXIIa, an example of positive feedback. In addition to amplifying its own generation by forming kallikrein, FXIIa (together with HMWK) proteolytically cleaves FXI, forming FXIa. In turn, FXIa (also bound to the charged surface by HMWK) proteolytically cleaves FIX to FIXa, which is also a protease **(Riddel *et al.*, 2007).**

FIXa and 2 downstream products of the cascade, FXa and thrombin, proteolytically cleave FVIII, forming FVIIIa, a cofactor in the next reaction. Finally, FIXa and FVIIIa together with Ca^{2+} (which may come largely from activated platelets) and negatively charged phospholipids (the major constituents of cell membranes) form a trimolecular complex termed tenase. Tenase then converts FX to FXa, yet another protease **(Riddel *et al.*, 2007).**

In a parallel series of interactions, FXa binds to the cofactor FVa, itself a downstream factor that participates in positive feedback with the present reaction to generate a complex with enzymatic activity known as prothombinase. This complex converts the proenzyme prothrombin to its enzyme form thrombin. Thrombin acts on fibrinogen to generate the fibrin monomer, which rapidly polymerizes to form the fibrin clot **(Fig 1) (Riddel *et al.*, 2007).**

B. Extrinsic pathway

The extrinsic pathway also includes protein cofactors and enzymes. This pathway is initiated by the formation of a complex between TF on cell surfaces and FVIIa that is located outside the vascular system. Nonvascular cells constitutively express the integral membrane protein TF (variably known as FIII or tissue thromboplastin), which is a receptor for the plasma protein FVII **(Riddel *et al.*, 2007).**

When an injury to the endothelium allows FVII to come into contact with TF, the TF nonproteolytically activates FVII to FVIIa. The mechanism of the initial conversion of the zymogen FVII to FVIIa is still debated but is most likely due to autocatalytic activation and not TF effect. This binding of FVIIa to TF forms an enzyme complex that activates FX to FXa. The FVIIa/TF complex, similar in function to the tenase complex, converts FX to its active form (FXa), which binds to the cofactor FV and is bound on membrane surfaces in the presence of calcium ions to generate the prothrombinase complex **(Riddel *et al.*, 2007).**

The prothrombinase complex converts prothrombin to thrombin, which converts fibrinogen to fibrin to generate the fibrin clot. During laboratory analysis of blood clotting, the extrinsic pathway of blood coagulation is evaluated using the prothombin time (PT). Regardless of whether FXa arises by the

intrinsic or extrinsic pathway, the cascade then proceeds along the common pathway **(Fig 1) (Riddel *et al.*, 2007).**

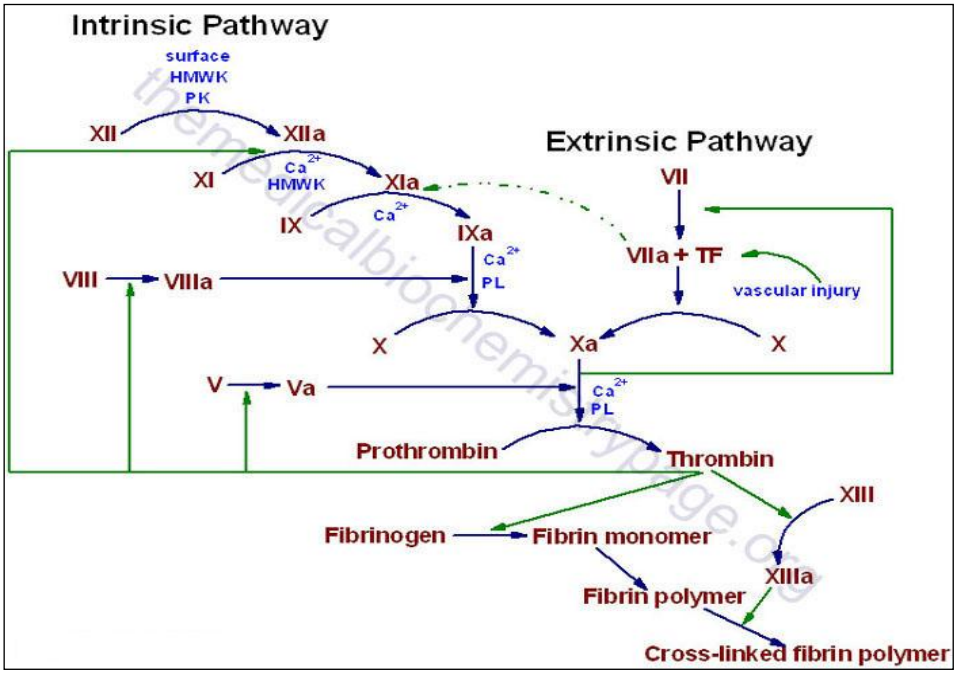

Fig. (1): Cascade model of coagulation.

C. Common pathway

The common pathway begins with the activation of FX within the intrinsic pathway, the extrinsic pathway, or both. FXa from either the intrinsic or extrinsic pathway is the first protease of the common pathway. FXa, in the presence of FV, Ca^{2+}, and phospholipids, converts prothrombin to its active form, thrombin. The main action of thrombin is to catalyze the proteolysis of the soluble plasma protein fibrinogen to form fibrin monomers that remain soluble. Fibrin monomers then

polymerize to form a gel of fibrin polymers that trap blood cells **(Fig 2) (Riddel *et al.*, 2007).**

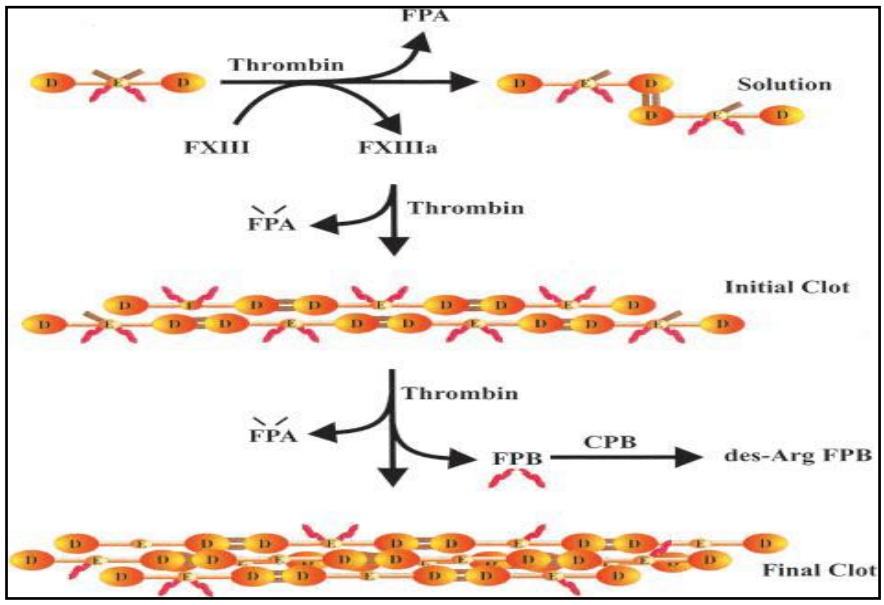

Fig. (2): The conversion of fibrinogen to fibrin.

When thrombin acts on the soluble fibrinogen monomer, it catalyzes release of fibrinopeptide A (FPA) and fibrinopeptide B (FPB) from the amino termini of the α and β chains of fibrinogen. This exposes polymerization sites in the E domains of fibrinogen such that noncovalent associations occur between the E and D domains of neighboring molecules, thereby producing double-stranded protofibrils. These laterally associate to form bundles, which continue to grow and branch, ultimately forming a three-dimensional web, which causes the solution to gel, thereby forming the familiar blood clot. The web is stabilized by covalent cross linkages between adjacent D domains, which are formed by factor XIIIa. A carboxypeptidase B (CPB)-like enzyme also removes the COOH terminal arginine from FPB ***(Nesheim, 2003).***

Thrombin also activates FXIII, which is converted to FXIIIa and mediates the covalent cross-linking of the fibrin polymers to form a mesh termed stable fibrin, which is less soluble than fibrin polymers. Thrombin can catalyze the formation of new thrombin from prothrombin and can catalyze the formation of the cofactors FVa and FVIIIa, resulting in

efficient amplification of coagulation. Because the common pathway contains the factors FX, FV, and FII (any deficiency in which may lead to a hemorrhagic disorder), these factors may be monitored by both the PT and the partial thromboplastin time (PTT) **(Fig 1) (Riddel *et al.*, 2007).**

II. ***Phases of coagulation according to the new cascade***

It is best to consider the highly interwoven array of physical, cellular, and biochemical processes that contribute to hemostasis as a series of process stages (phases) rather than pathways. The phases of initiation, propagation, and termination illustrate the intricate processes involved in the maintenance of vascular integrity **(Fig 3) (Table 1) (Riddel *et al.*, 2007).**

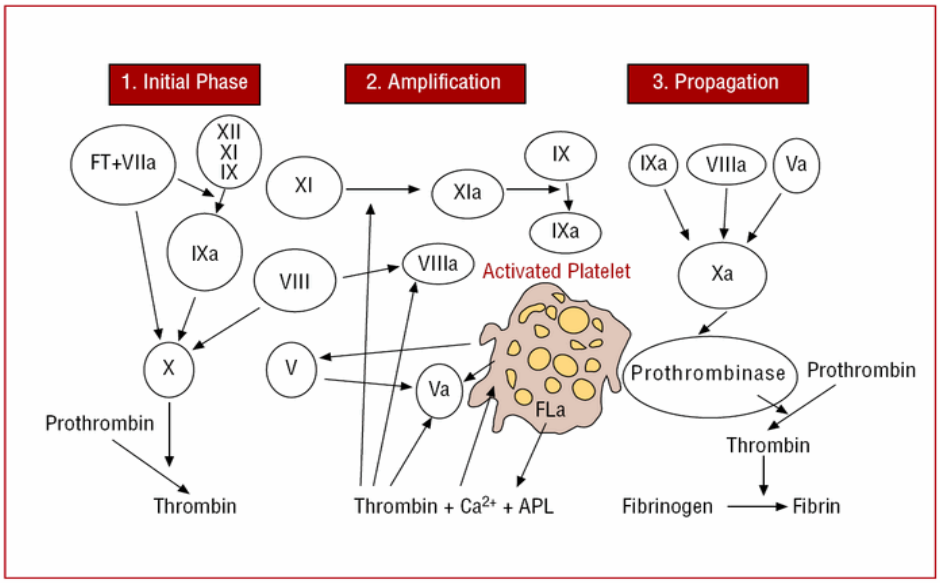

Fig. (3): Phases of coagulation according to the new cascade.

Table (1): Summary of the 4 phases of coagulation, as proposed by the current cell-based theory of coagulation ***(Riddel et al., 2007).***

Initiation	Amplification	Propagation	Termination
Vascular endothelium and circulating blood cells are perturbed; interaction of plasma-derived FVIIa with tissue factor	Thrombin activates platelets, cofactors FVa and FVIII on the platelet surface, and FXI on the platelet surface	Results in the production of a significant level of thrombin activity, generation of a stable plug at the site of injury, and cessation of blood loss	Clotting process is limited to avoid thrombotic occlusion in surrounding normal areas of the vasculature

III. Regulation of blood coagulation

The regulation of blood coagulation is essential to avoid a generalized activation of the system and massive fibrin deposition. To be effective, the system must be active only at a local site of vascular injury and must remain active only for a sufficient period of time to produce enough fibrin to seal the wound. To achieve this, a number of regulatory mechanisms are in place. Each coagulation protein is sequentially activated, so that only a small proportion of an active serine protease is available at any given time **(Norris, 2003).**

Second, coagulation activation and formation of the complexes can proceed only on the surface of activated cells and platelets—where negatively charged phospholipids are exposed and the appropriate receptors are available for binding. Similarly, the essential trigger for the cascade, tissue factor, is

available only on the surface of activated monocytes and cells which are exposed to plasma only as a result of vascular injury **(Norris, 2003).**

The most important regulatory pathways, however, are a series of anticoagulant proteins and cofactors which bind to activated coagulation factors and limit their period of activity. Finally, in the presence of fibrin, fibrinolysis is stimulated which dissolves fibrin into fibrin degradation products **(Norris, 2003).**

A. Tissue factor pathway inhibitor (TFPI)

The initial trigger for coagulation activation is the TF-VIIa complex which is a potent activator of both factor IX and X. For this reason, the activity of this complex must be quickly inactivated. This is achieved by a specific inhibitor of the complex, TFPI released constitutively from endothelial cells. Initially it forms a complex with Xa through one of its domains and the complex can then bind to the TF-VIIa complex with another binding domain to form a quaternary complex. The effect is rapid inhibition of extrinsic activation of the cascade. In addition TFPI also promotes the internalization and subsequent degradation of the inhibited complex **(Norris, 2003).**

B. Antithrombin

Antithrombin is a serine protease inhibitor that can inhibit many of the activated coagulation enzymes. Key components of the cascade, such as factors IXa, Xa, TF-VIIa complex and thrombin, are rapidly bound by antithrombin and neutralized. The ability of antithrombin to inhibit these factors is greatly accelerated by heparin sulphate proteoglycans and this is the basis for the anticoagulant action of the pharmaceutical heparins. Thrombin bound to antithrombin forms the stable thrombin–antithrombin (TAT) complex, which is rapidly cleared from the circulation **(Norris, 2003).**

C. The protein C anticoagulant pathway

The protein C anticoagulant pathway is thought to be the major mechanism by which thrombosis in the microcirculation is prevented. Protein C is a vitamin-K-dependent plasma protein which circulates as an inactive zymogen. The activation of protein C also requires thrombin. Free thrombin must first bind to another transmembrane protein, thrombomodulin which transfer thrombin to the endothelium where the endothelial protein C receptor (EPCR) presents protein C for activation. Thrombin complexed to thrombomodulin activates protein C, possibly through a conformational change in the thrombin enzyme. Activated protein C (APC) is inactive as an inhibitor while it remains bound to the EPCR. To become active, APC

must dissociate from EPCR and bind to its cofactor protein S. The protein S/APC complex can inactivate both factor Va and factor VIIIa, effectively shutting down prothrombinase and tenase activity respectively **(Norris, 2003).**

D. Inhibitors of the intrinsic system

The major inhibitor of the intrinsic system is C1-inhibitor, which inhibits factor XIIa. Binding of C1 inhibitor irreversibly inactivates at least 90% of XIIa. α1-Antitrypsin can inhibit factor XIa and acts in a similar manner to antithrombin, forming a stable complex with its target serine protease. α 2-Macroglobulin is a broad-spectrum proteinase inhibitor which acts as a secondary inhibitor of many proteinases involved in the coagulation cascade, including kallikrein and thrombin and the fibrinolytic enzyme plasmin **(Norris, 2003).**