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Molecular study of Thrombosis in a Sample of Iraqi Patients

A Thesis

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Summary

This study aimed to investigate the association of F5 gene single nucleotide polymorphism (SNP) with the incidence of thrombosis. Blood samples patientsduring the period from November 2014 to January ٤٠werecollected from Hospital), Kadhimiya Hospital, of (Yarmook (C.C.U) , from critical care unit٥201 as well as from 10unrelated healthy control group.

This study found the age group between 50 to 60 are more susceptible to thrombosis 45% and the thrombosis was more frequent in male 55% from female (DNA) was extracted from Deoxyribonucleic acid 45% the significant ($p<0.01$). whole blood samples,whereas, serum samples were analyzed using troponin test (TNT) for detection of thrombosis.

Polymerase chain reaction (PCR) was achieved on extracted DNAusing eleven with product size specific primers for F5 gene:the first primer (Fve3) third primer (Fve6)with (310bp), with product size (Fve4) (228bp),second primer product size(547bp),fourthprimer (Fve7) with product size (241bp),fifth primer (Fve8)with product size(306bp),sixth primer (Fve12) with product size(286bp), seventh primer (Fve13a)with product length (260bp) ,eighth primer (Fve13c) with primer product size (317bp) ,ninthprimer (Fve15) with product size (600bp) ,tenth (Fve16)with product size (333bp) and eleventh primer (Fve25)with product size(390bp).

Result found to be change in DNA .PCR products of F5 gene were sequenced which where mostly SNP. This change was in three types: substitution 24.86%, insertion 28.57% and deletion 28.57%, a Leiden mutation was also identified among patients.

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APC	activated protein C
APTT	activated partial thromboplastin time
Bp	base pair
CVST	Cerebral venous sinus thrombosis
DIC	Disseminated intravascular coagulation

DVT	Deep vein thrombosis
F5 gene	Factor V gene
MI	Myocardial infarction
MTHFR	methylene tetrahydrofolate reductase
NBTE	nonbacterial thrombotic endocarditis
OLT	orthotropic liver transplantation
PC	protein C
PCR	polymerase chain reaction
PPP	Pentose Phoshate Pathway
PS	protein S
PT	prothrombin time
PVT	Portal vein thrombosis
Rpm	round per minute
SERTs	sensitive 5-HT transporters
SNP	single nucleotide polymorphism

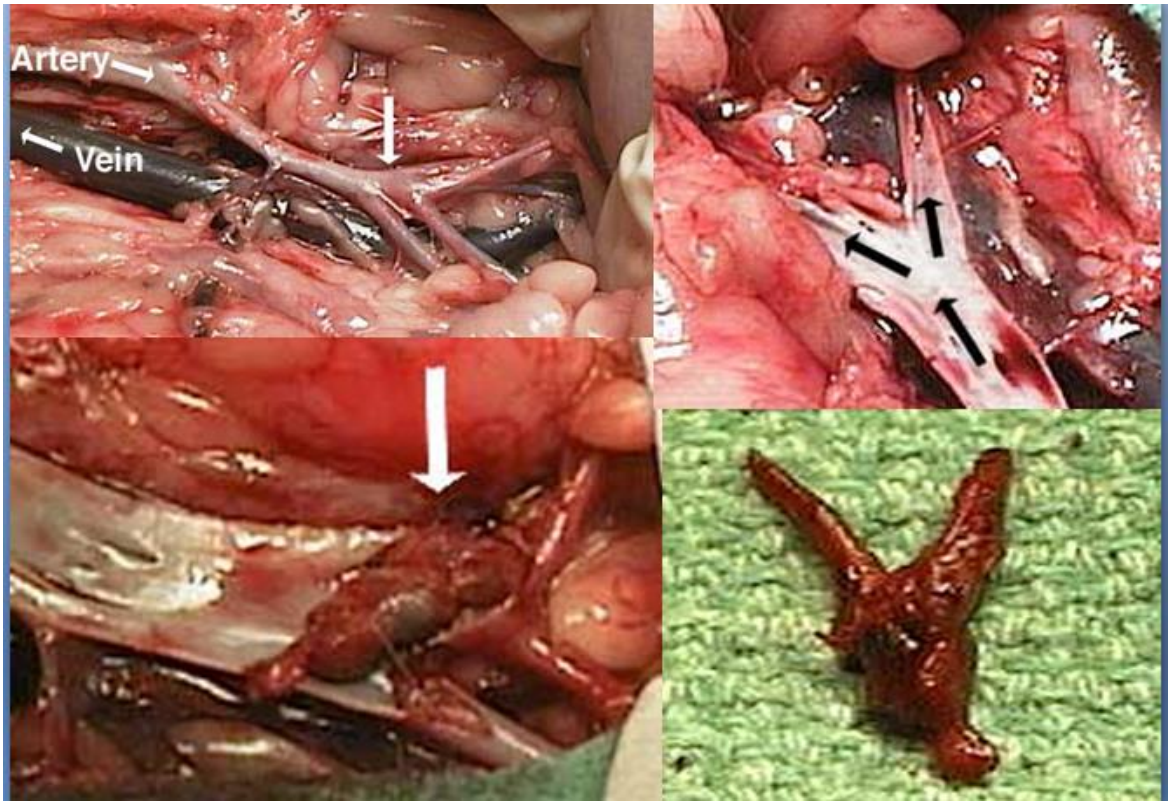
TIPS	Transjugular intrahepatic portosystemic shunt
TT	thrombin time
UV	Ultra violate
V	Volt
VMAT	vesicular monoamine transporter
VTE	venous thromboembolism

1. Introduction and Literature Review.

.1.1.Introduction

A thrombus, or colloquially a blood clot, is the final product of the blood coagulation step in hemostasis. There are two components to a thrombus: and a mesh of cross- , aggregated platelets that form a platelet plug
The substance making up a thrombus is sometimes .linked fibrin protein called cruor. A thrombus is a healthy response to injury intended to prevent bleeding, but can be harmful in thrombosis, when clots obstruct blood flow Saladin and Kenneth , 2012).(through healthy blood vessels
Mural thrombi are thrombi that adhere to the wall of a blood vessel. They occur in large vessels such as heart and aorta, and can restrict blood flow but usually do not block it entirely. They appear grey-red with alternating light and dark lines (known as lines of Zhan) which represent bands of fibrin (lighter) with entrapped white blood cells and red blood cells (darker) (Kumar *et al*., 2007).
(DIC) involves widespread microDisseminated intravascular coagulation thrombi formation throughout the majority of the blood vessels. This is due to excessive consumption of coagulation factors and subsequent activation of fibrinolysis using all of the body's available platelets and clotting factors. The end result is hemorrhaging and ischaemic necrosis of tissue/organs. Causes are septicemia, acute leukemia, shock, snake bites, fat emboli from broken bones, or other severe traumas. DIC may also be seen in pregnant females. Treatment involves the use of fresh frozen plasma to restore the level of clotting factors in the blood, platelets and heparin to prevent further thrombi formation (Ungprasert *et*).٢*al.*, 201
).

Thoracic aortic thrombus is a rare pathology that usually originates from an atherosclerotic aortic wall lesion or an aortic aneurysm and is a potential source of visceral, cerebral and peripheral embolism as shown in figure (1-1) .



The thrombus when we open up the arteries (Heart Diseases and Their : Figure (1- 1) .Treatment , 2015)

Aim of the study:

1. Identification of factors affecting thrombosis related to genetic and body physiology.
2. Identification the differences in gene between patients with thrombosis and healthy control by using PCR analysis.
3. Compared the sequencing of PCR product regarding F5 gene for all patients groups.
4. Prevalence of factor V Leiden mutation in patients with thrombosis in Iraq.

1.2. Literature Review.

. 1.2.1. Thromboembolic Disease

Many developments during the past decade have led to a greatly improved understanding of the etiology of abnormal blood clotting; improvements in clinical diagnosis and therapy have been reported (Thorneycroft and Goldzieher, 2003) . Blood clots can develop in the arterial circulation (arterial thrombosis) or venous circulation (venous thrombosis) . Arterial thrombi usually develop in arteries diseased by the process of atherosclerosis. The factors that lead to venous thrombosis are less well understood, but inactivity and small injuries to the veins may play a role (Kearon, 2003).

Blood clots that form in the circulation often break off and travel to other areas of the circulation, where they can cause major organdamage or death. Heart attacks and strokes can be caused by these emboli. Emboli that lodge in the lungs (pulmonary emboli) can rapidly cause death. Since thrombosis and embolism occur together, the process is usually referred to as thromboembolism (Dimitrova *et al*., 2013) .

Most individuals who develop thromboembolism have one or more risk factors. Many of the non-inherited (acquired) risk factors have been recognized for decades, but a rapid series of scientific discoveries over the past two decades have led to the recognition of numerous inherited (genetic) factors that can increase an individual's risk of developing thromboembolic disease. These inherited factors act in conjunction with acquired risk factors and involve the vessels, blood flow through the vessels, and the blood platelets and chemicals in the blood that are part of the hemostatic system (Roger *et al*., 2003).

Thrombophilia refers to individuals who have a tendency to develop thrombosis from either acquired or inherited causes, or both. In view of the enormous amount

of medical resources needed to care for patients with thromboembolic disease, there is a great interest in avoiding acquired risk factors and the identification and early treatment of patients who have a high risk of developing disease. (Roger *et al*., 2003).

. Morphology.1.2.2

Thrombi can develop anywhere in the cardiovascular system (e.g., in cardiac chambers, on valves, or in arteries, veins, or capillaries). The size and shape of a thrombus depend on the site of origin and the cause. Arterial or cardiac thrombi typically begin at sites of endothelial injury or turbulence; venous thrombi characteristically occur at sites of stasis (James, 2010).

Thrombi are focally attached to the underlying vascular surface; arterial thrombi tend to grow in a retrograde direction from the point of attachment, while venous thrombi extend in the direction of blood flow (thus both tend to propagate toward the heart). The propagating portion of a thrombus tends to be poorly attached and therefore prone to fragmentation, generating an embolus (Lüscher *et al*. ,2007).

Thrombi can have grossly and microscopically apparent laminations called lines of Zahn these represent pale platelet and fibrin layers alternating with darker erythrocyte- rich layers. Such lines are significant only in that they represent thrombosis in the setting of flowing blood; their presence can therefore potentially distinguish antemortem thrombosis from the bland non laminated clots that occur in the postmortem state . Although such lines are typically not as apparent in veins or smaller arteries (thrombi formed in sluggish venous flow usually resemble statically coagulated blood), careful evaluation generally reveals ill- defined laminations (Lee *et al*., 2012).

1.2.2.1.Mural thrombi .

dilated Abnormal myocardial contraction (resulting from arrhythmias, cardiomyopathy, or myocardial infarction) or endomyocardial injury (caused by myocarditis, catheter trauma) promotes cardiac mural thrombi . While ulcerated atherosclerotic plaques and aneurismal dilation promote aortic thrombosis (Furie *et al*., 2008).

1.2.2.2.Arterial thrombi .

Are frequently occlusive and are produced by platelet and coagulation they are typically a friable meshwork of platelets, fibrin, erythrocytes, activation and degenerating leukocytes. Although arterial thrombi are usually superimposed on an atherosclerotic plaque, other vascular injury (vasculitis, trauma) can be). *et al*., 2005 Hatzinikolaou(involved

1.2.2.3.Venous thrombosis (phlebothrombosis).

Is almost invariably occlusive, and the thrombus can create a long cast of the , venous thrombosis is largely the result of activation of the coagulation lumen cascade, and platelets play a secondary role. Because these thrombi form in the sluggish venous circulation, they also tend to contain more enmeshed erythrocytes and are therefore called red, or stasis, thrombi. The veins of the lower extremities are most commonly affected (90% of venous thromboses); however, venous thrombi can occur in the upper extremities, periprostatic plexus, or ovarian and periuterine veins; under special circumstances, they may be found in the dural sinuses, portal vein, or hepatic vein (Jahangir, 2008).

1.2.2.4.Postmortem clots.

Can sometimes be mistaken at autopsy for venous thrombi. However, postmortem "thrombi" are gelatinous, with a dark red dependent portion where red cells have settled by gravity, and a yellow "chicken fat" supernatant, and they are usually not attached to the underlying wall. In contrast, red thrombi are firmer and

are focally attached, and sectioning reveals strands of gray fibrin (Hoffman *et al*., 2000).

1.2.2.5.Vegetations.

Bacterial or fungal blood- borne infections can cause valve damage, subsequently leading to large thrombotic masses (infective endocarditis), sterile vegetations can also develop on non infected valves in hypercoagulable states (Miyata *et al*.,2007).

1.2.2.6.nonbacterial thrombotic endocarditis (NBTE).

Less commonly, is a form of endocarditis in which small sterile vegetations are deposited on the valve leaflets, sterile, verrucous endocarditis can occur in the setting of systemic lupus erythematosus (Milind , 2008).

1.2.3. Classification of thrombosis .

There are two distinct forms of thrombosis, venous thrombosis and arterial thrombosis, each of which can be presented by several subtypes (Brill *et al*., 2011).

Venous thrombosis.١.3.١.٢.

- **Venous thrombosis:** Is the formation of a thrombus (blood clot) within a vein. There are several diseases which can be classified under this category (Lederle *et al*., 2011).
- **Deep vein thrombosis (DVT):** Is the formation of a blood clot within a deep vein. It most commonly affects leg veins, such as the femoral vein. Three factors are important in the formation of a blood clot within a deep vein ,these are the rate of blood flow, the thickness of the blood and qualities of the vessel wall. Classical signs of DVT include swelling, pain and redness of the affected area (Saha *et al.*, 2011).
- **Portal vein thrombosis (PVT):** Affects the hepatic portal vein, which can lead to portal hypertension and reduction of the blood supply to the liver. It usually

has a pathologic al cause such pancreatitis, cirrhosis, diverticulitis or cholangiocarcinoma Renal vein thrombosis: is the obstruction of the renal vein by a thrombus. This tends to lead to reduced drainage from the kidney. Anticoagulation therapy is the treatment of choice (Webster *et al.*,2005).

- **Jugular vein thrombosis :** Is a condition that may occur due to infection, intravenous drug use or malignancy. Jugular vein thrombosis can have a varying list of complications, including: systemic sepsis, pulmonary embolism, and papilledema. Though characterized by a sharp pain at the site of the vein, it can prove difficult to diagnose, because it can occur at random (Dale , 2005).
- **Budd-Chiari syndrome :** Is the blockage of the hepatic vein or the inferior vena cava. This form of thrombosis presents with abdominal pain, ascites and hepatomegaly. Treatment varies between therapy and surgical intervention by the use of shunts (Rajani *et al*.,2009).
- **Paget-Schroetter disease :** Is the obstruction of an upper extremity vein (such as the axillary vein or subclavian vein) by a thrombus. The condition usually comes to light after vigorous exercise and usually presents in younger, otherwise healthy people. Men are affected more than women **(**Flinterman *et al*., 2008).
- **Cerebral venous sinus thrombosis (CVST) :** Is a rare form of stroke which results from the blockage of the dural venous sinuses by a thrombus. Symptoms may include headache, abnormal vision, any of the symptoms of stroke such as weakness of the face and limbs on one side of the body and seizures. The diagnosis is usually made with a CT or MRI scan. The majority of persons affected make a full recovery (Canhão *et al*., 2005).
- **Cavernous sinus thrombosis** : Is a specialized form of cerebral venous sinus thrombosis, where there is thrombosis of the cavernous sinus of the basal skull dura, due to the retrograde spread of infection and endothelial damage from

the danger triangle of the face. The facial veins in this area anastomose with the superior and inferior ophthalmic veins of the orbit, which drain directly posteriorly into the cavernous sinus through the superior orbital fissure. *Staphylococcal* or *Streptococcal* infections of the face, for example nasal or upper lip pustules may thus spread directly into the cavernous sinus, causing stroke-like symptoms of double vision, squint, as well as spread of infection to cause meningitis (Sahjpaul and Lee ,1999).

.3.2. Arterial thrombosis.١.٢

Is the formation of a thrombus within an artery. In most cases, arterial thrombosis follows rupture of atheroma, and is therefore referred to as athero thrombosis. another common cause of arterial occlusion is atrial fibrillation, which causes a blood stasis within the atria with easy thrombus formation. In addition, it is well known that the direct current cardio version of atrial fibrillation carries a great risk of thrombo embolism, especially if persisting more than 48 hours. Thrombo embolism strikes approximately 5% of cases not receiving anticoagulant therapy. When cardiac rhythm is restored clots are pushed out from atria to ventricles and from these to the aorta and its branches. Arterial thrombosis can embolism and is a major cause of arterial embolism, potentially causing infarction of almost any organ in the body (Hatzinikolaou *et al.*, 2005).

- **Stroke:** Is the rapid decline of brain function due to a disturbance in the supply of blood to the brain. This can be due to ischemia, thrombus, embolus (a lodged particle) or hemorrhage (a bleed). In thrombotic stroke, a thrombus (blood clot) usually forms around atherosclerotic plaques. Since blockage of the artery is gradual, onset of symptomatic thrombotic strokes is slower. Thrombotic stroke can be divided into two categories large vessel disease and small vessel disease (Glynn *et al.*, 2007) .

The former affects vessels such as the internal carotids, vertebral and the circle of Willis. The latter can affect smaller vessels such as the branches of the circle of Willis .Myocardial infarction (Sean *et al*., 2008).

- **Myocardial infarction (MI) or heart attack:** Is caused by ischemia, (restriction in the blood supply), often due to the obstruction of a coronary artery by a thrombus. This restriction gives an insufficient supply of oxygen to the heart muscle which then results in tissue death,(infarction). A lesion is then formed which is the infarct. MI can quickly become fatal if emergency medical treatment is not received promptly. If diagnosed within 12 hours of the initial episode (attack) then thrombolytic therapy is initiated (Steg *et al*.,2012).
- **Other sites :** Hepatic artery thrombosis usually occurs as a devastating complication after liver transplantation. An arterial embolus can also form in the limbs (Bekker *et al*., 2009).

1.2.4. Mechanisms of thrombus formation.

Hemostasis is the process that maintains the integrity of a closed, high-pressure circulatory system after vascular damage. Vessel-wall injury and the extravasations of blood from the circulation rapidly initiate events in the vessel wall and in blood that seal the breach. Circulating platelets are recruited to the site of injury, where they become a major component of the developing thrombus; blood coagulation, initiated by tissue facto culminates in the generation of thrombin and fibrin. These events occur concomitantly, and under normal conditions, regulatory mechanisms contain thrombus formation temporally and spatially (Borissoff *et al*., 2011).

When pathologic processes overwhelm the regulatory mechanisms of hemostasis, excessive quantities of thrombin form, initiating thrombosis.

Thrombosis is a critical event in the arterial diseases associated with myocardial infarction and stroke, and venous thromboembolic disorders account for considerable morbidity and mortality. Moreover, venous thrombosis is the

second leading cause of death in patients with cancer. Platelet activation can occur through different mechanisms such as a vessel wall breach that exposes collagen, or tissue factor encryption. The platelet activation causes a cascade of further platelet activation to eventually causing the formation of the thrombus, this process is regulated through thrombo regulation (Bruce *et al*., 2008).

Thrombo regulation is the series of mechanisms in how a primary clot is regulated. These mechanisms include, competitive inhibition or negative feedback. It includes primary hemostasis, which is the process of how blood platelets adhere to the endothelium of an injured blood vessel (Ruiz and Guillermo ,2009).

Platelet aggregation is fundamental to repair vascular damage and the initiation of the blood thrombus formation ,the elimination of clots is also part of thromboregulation. Failure in platelet clot regulation may cause hemorrhage or thrombosis. The Substances called thrombo regulators control every part of these events (Brass and Lawrence , 2003).

1.2.4.1. Primary hemostasis inducers.

One primary function of thromboregulation is the control of primary hemostasis, which is the platelet aggregation process. Some thrombo regulators enhance platelet aggregation and some others inhibit the process. Platelet aggregation plays a critical role in the genesis of a resulting thrombus. Adhesion should remain local, but platelet aggregation must grow exponentially to form a platelet thrombus and prevent blood loss. Platelet aggregation factors are the regulators that enhance the adhesion and stimulate the platelets to secrete its granules. It has been shown that collagen, exposed after the injury to the endothelial cover of the vessel, plays as an agonist in platelet adhesion and its activation (Furie *et al*., 2005).

The binding of platelets to the sub-endothelial collagen stimulates the secretion of ADP, TXA2, and serotonin present in the platelet granules. ADP-dependent aggregation is mediated by two receptors: the purinergic P2Y1, coupled to Gαq,

mediates the shape in the structure of platelets and triggers the aggregation process (Marcus *et al*.,1993).

Thromboxane A2 (TX2) has a positive feedback in platelet activation. It is produced by the oxygenation of arachidonic acid by two enzymes, cycloxygenase and thromboxane A2 synthase. TX2 effects are mediated by G protein-coupled receptors, subtypes TPα and TPβ. Both receptors mediate phospholipase C stimulation causing an increase of intracellular levels of inositol 1,4,5-triphosphate and diacylglycerol. Inositol 1,4,5-triphosphate causes an increase in Ca concentration and the release of diacylglycerol activates PKC (Iyú *et al*.,2010).

TPα stimulates cAMP levels whereas TPβ inhibits the level of intracellular cAMP. Serotonin, 5-HT, is an amine synthesized in the gut and it is released into the bloodstream after the activation of presynaptic neurons or enterochromaffin cells stimulation. Later, it is sequestered by the platelets using antidepressant-sensitive 5-HT transporters (SERTs) and into platelet's granules by the vesicular monoamine transporter (VMAT). After the secretion, 5-HT increases the effects of pro thrombotic agents by its binding with 5-HT2 receptors (Ruiz and Guillermo , 2009).

1.2.4.٢. Primary hemostasis inhibitors.

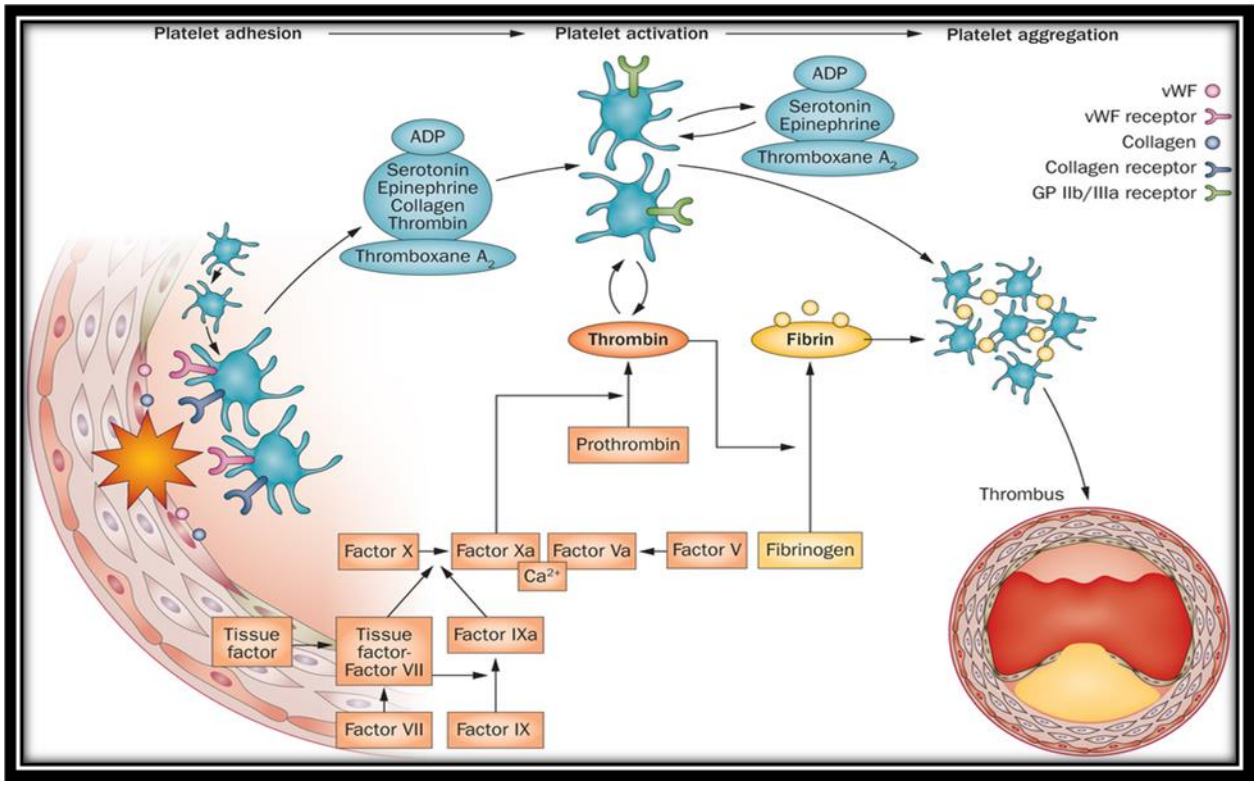
Thrombo regulation is also in charge of regulating the process of clot elimination,

called primary hemostasis inhibition. These inhibitors are substances that prevent the clot formation by preventing platelet adhesion . Platelet inhibition is important to prevent thrombotic episodes or the formation of blood clot and consequently preventing heart attacks and strokes. Some primary hemostasis inhibitors are cAMP, prostacyclin, PGE1, and kistrin. cAMP, cyclic adenosine monophosphate, phosphorylate messengers via protein kinase A (PKA) (Jüttner *et al*., 2010).

These signaling elements include thromboxane A2, receptor type α, phospholipase Cβ3, and IP3 receptors. Signalization in platelets is very sensitive in cAMP levels. Nitric oxide (NO) stimulates cGMP production and therefore the activation cGMP-dependent protein kinase (G kinase). This kinase inhibits Gαq-phospholipase C-inositol 1,4,5-triphosphate signaling and the mobilization of calcium inside the cell for thromboxane A2. PGI2, prostacyclin, binds to IP receptors that catalyze cAMP formation. This process is mediated via GTP-binding protein Gs and adenylyl cyclase. PGE1 binds to IP receptors. IP receptors bind with ionophores that induce ADP and serotonin secretion. PGE1 inhibits the secretion of factors that stimulate platelet aggregation by competitive inhibition. Kistrin is a protein inhibitor of platelet aggregation. It belongs to the homologous family of glycoprotein IIb-IIa antagonists. Kistrin has an adhesion site that binds to GP IIb-IIIa (Adler *et al*., 1991).

Plaque rupture exposes sub endothelial components. Platelet adhesion during the rolling phase is mediated by interactions between vWF and GP Ib/V/IX receptor complexes located on the platelet surface, and between platelet collagen receptors (GP VI and GP Ia) and collagen exposed at the site of vascular injury. Binding of collagen to GP VI induces the release of activating factors (ADP, thromboxane A2, serotonin, epinephrine, and thrombin) ,which promote interactions between adherent platelets, as well as further recruitment and activation of circulating platelets as shown in figure (1-2) (Bruce *et al*., 2008). hape, expression of SPlatelet activation leads to changes in platelet proinflammatory molecules, platelet procoagulant activity, and activation of platelet integrin GP IIb/IIIa. Activated GP IIb/IIIa binds to the extracellular ligands fibrinogen and vWF, leading to platelet aggregation and thrombus formation. Vascular injury also exposes subendothelial tissue factor, which forms a complex with factor VIIa and sets off a chain of events that culminates in formation of the

prothrombinase complex. Prothrombin is converted to thrombin, which subsequently converts fibrinogen to fibrin, generating a fibrin-rich clot. Abbreviations: GP, glycoprotein; vWF, von Willebrand factor .



Mechanism of thrombus formation.: Figure (1-2)

1.2.5.Pathophysiology.

A thrombus occurs when the hemostatic process, which normally occurs in response to injury, becomes activated in an uninjured or slightly injured vessel. A thrombus in a large blood vessel will decrease blood flow through that vessel (termed a mural thrombus) as shown in figure (1-3) (Waldo, 2008) . In a small blood vessel, blood flow may be completely cut off (termed an occlusive thrombus), resulting in death of tissue supplied by that vessel (Bedard *et al*., 2008).

Some of the conditions which elevate risk of blood clots developing include atrial fibrillation (a form of cardiac arrhythmia), heart valve replacement, a recent heart attack (also known as a myocardial infarction), extended periods of inactivity (see deep venous thrombosis), and genetic or disease-related deficiencies in the blood's clotting abilities (Lyaker *et al*.,2013).

Thrombus can block the flow of blood through a vein or artery. If it detaches from the vessel wall and lodges in the lungs or other vital organs, it can become a life-threatening embolus (Engl , 2008).

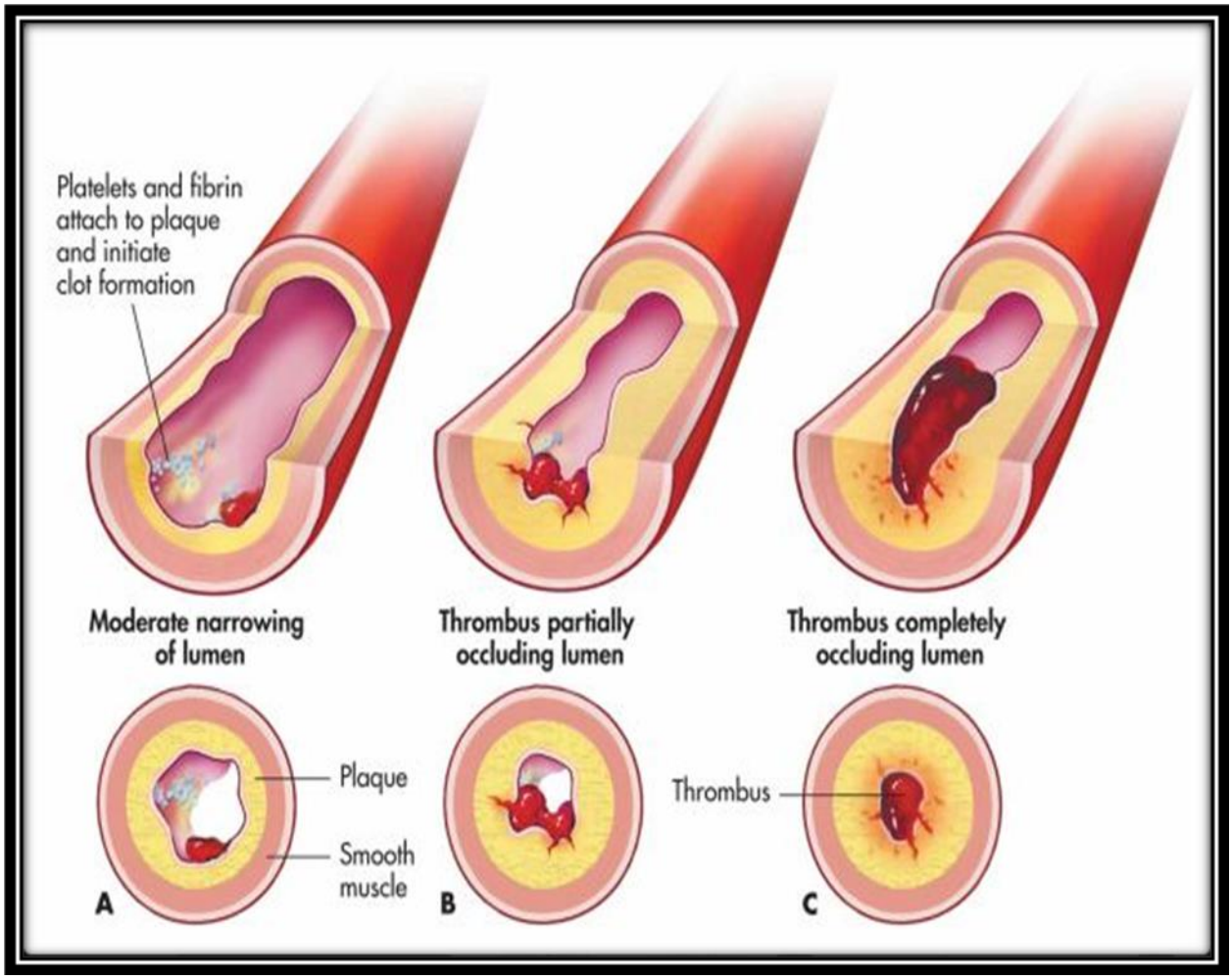
A pathological thrombus forms when there is an imbalance in the blood coagulation system (Furie , 2008).

Haemostasis is necessary for survival, but the pathological formation of a thrombus poses significant health risks (Tapson and Engl , 2008).

The coagulation system depends on a delicate balance between:

- Natural coagulant and anticoagulant factors.
- The coagulation and fibrinolytic system.

An imbalance in these systems can result in pathological coagulation. The of resulting thrombus can potentially obstruct blood flow, leading to a number attacks and cardio embolic stroke in heart including conditions health serious patients with atrial fibrillation, and venous thromboembolism (Fuster *et al* ., 2005). Venous thromboembolism can manifest as deep vein thrombosis and /or pulmonary embolism two distinct but related aspects of the same disease .



Figure(1-3):Thrombus formation in an atherosclerotic vessel ,Thrombus formation in an atherosclerotic vessel depicting (A) the initial clot formation, and (B) and (C) the varying degrees of . occlusion

There are three primary influences on thrombus formation (called Virchow's triad):**(1)** endothelial injury, **(2)** stasis or turbulence of blood flow, and **(3)** blood hypercoagulability (Asselta *et al*., 2006).

1.2.5.1. Endothelial Injury.

This is a dominant influence, since endothelial loss by itself can lead to thrombosis. It is particularly important for thrombus formation occurring in the heart or in the arterial circulation, where the normally high flow rates might otherwise hamper clotting by preventing platelet adhesion or diluting coagulation 2009). factors (Shivakumar *et al*.,

Thrombus formation within the cardiac chambers (e.g., after endocardial injury due to myocardial infarction), over ulcerated plaques in atherosclerotic arteries, or at sites of traumatic or inflammatory vascular injury (vasculitis) is largely a function of endothelial injury. Clearly, physical loss of endothelium leads to exposure of sub endothelial, adhesion of platelets, release of tissue factor, and local depletion of PGI2 and plasminogen activators. However, it is important to note that endothelium need not be denuded or physically disrupted to contribute to the development of thrombosis; any perturbation in the dynamic balance of the prothrombotic and antithrombotic activities of endothelium can influence local clotting events Thus, dysfunctional endothelium may elaborate greater amounts of procoagulant factors (e.g., platelet adhesion molecules, tissue factor, plasminogen activator inhibitors) or may synthesize fewer anticoagulant effectors (e.g., thrombomodulin, PGI2, t-PA) (Varga *et al* ., 2009).

Significant endothelial dysfunction (in the absence of endothelial cell loss) may occur with hypertension, turbulent flow over scarred valves, or by the action of bacterial endotoxins. Even relatively subtle influences, such as homocystinuria, hypercholesterolemia, radiation, or products absorbed from cigarette smoke, may be sources of endothelial dysfunction (Clin , 2006).

1.2.5.2. Alterations in normal blood flow.

Turbulence contributes to arterial and cardiac thrombosis by causing endothelial injury or dysfunction, as well as by forming countercurrents and local pockets of stasis; stasis is a major contributor to the development of venous thrombi. Normal blood flow is laminar, such that platelets flow centrally in the vessel lumen, separated from the endothelium by a slower moving clear zone of plasma. Stasis and turbulence therefore: Disrupt laminar flow and bring platelets into contact with the endothelium Prevent dilution of activated clotting factors by fresh flowing blood Retard the inflow of clotting factor inhibitors and permit the buildup of thrombi Promote endothelial cell activation, resulting in local thrombosis, leukocyte adhesion, Turbulence and stasis contribute to thrombosis in several clinical settings (Huang *et al* ., 2010).

Ulcerated atherosclerotic plaques not only expose sub endothelial but also cause turbulence. Abnormal aortic and arterial dilations, called aneurysms, create local stasis and consequently a fertile site for thrombosis Acute myocardial infarction results in focally no contractile myocardium; ventricular remodeling after more remote infarction can lead to aneurysm formation. In both cases cardiac mural thrombi form more easily because of the local blood stasis Mitral valve stenosis (e.g., after rheumatic heart disease) results in left atrial dilation. In conjunction with atrial fibrillation, a dilated atrium is a site of profound stasis and a prime location for development of thrombi (Chang , 2004).

Hyper viscosity syndromes (such as polycythemia) increase resistance to flow and cause small vessel stasis; the deformed red cells in sickle cell anemia cause vascular occlusions, with the resultant stasis also predisposing to thrombosis (Mackman *el at*., 2008).

1.2.5.3. Hypercoagulability.

Hypercoagulability generally contributes less frequently to thrombotic states but is nevertheless an important component in the equation. It is loosely defined as any alteration of the coagulation pathways that predisposes to thrombosis, and it can be divided into primary (genetic) and secondary (acquired) disorders. Hypercoagulabl States (Lip GYH *et al*., 2000).

1- Primary (Genetic)

- Common

Mutation in factor V gene (factor V Leiden)

Mutation in prothrombin gene

Mutation in methyltetrahydrofolate gene

- Rare

Antithrombin III deficiency

Protein C deficiency

Protein S deficiency

- Very rare

Fibrinolysis defects

2- Secondary (Acquired)

- High risk for thrombosis

rest or immobilization Prolonged bed

Myocardial infarction

Atrial fibrillation

Tissue damage (surgery, fracture, burns)

Cancer

Prosthetic cardiac valves

Disseminated intravascular coagulation

Heparin-induced thrombocytopenia

Antiphospholipid antibody syndrome (lupus anticoagulant syndrome)

- Lower risk for thrombosis

Cardiomyopathy

Nephrotic syndrome

estrogenic states (pregnancy) Hyper

Oral contraceptive use

Sickle cell anemia

Smoking

as shown in figure (1-4) (Bayer , 2012).

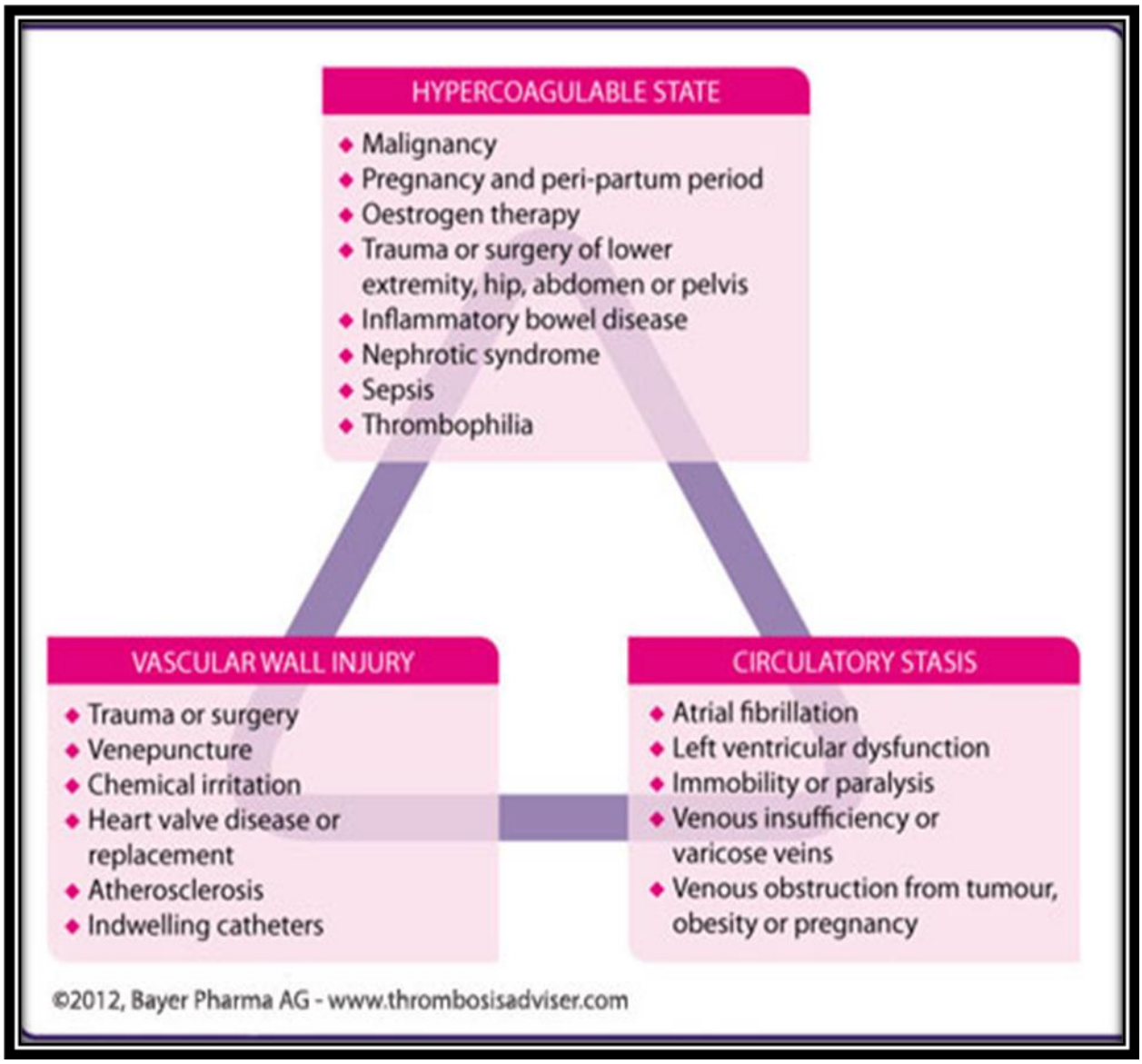


Figure (1-4) : hypercoagulability of pathophysiology .

. Diagnosis of Thromboembolic.٦1.2.

The recent identification of inherited and acquired risk factors for thrombosis greatly improved the ability of doctors to diagnose thrombotic disorders and has identify individuals at increased risk of thrombosis. There is a debate regarding whom should be tested for thromboembolic disease, but most doctors feel that ndividuals with a family history of abnormal blood clotting, as well as those with i well-defined acquired risk factors, should be screened for the antiphospholipid

. (Bauer *et al*., 2002) antibody syndrome and the common inherited risk factors This testing can help in estimating the risk of thromboembolic disease and planning long-term anti-thrombotic management. The coagulation laboratory performs tests that detect the function and amount of various coagulation factors, while the molecular diagnosis laboratory looks for specific abnormalities in DNA. Generally, coagulation tests are used to screen for the presence of disease and to monitor treatment, while the molecular assays are used for confirmation. Since the coagulation system is disrupted by the body's reaction to thromboembolic disease, coagulation screening in a person who suffers from a blood clot should be delayed for several weeks after discharge from the hospital and the coagulation system returns to a "steady state. The molecular assays, however, are not affected by drugs , 2010). Zhonghua(or disease processes and can be performed at any time

In a person with a suspected inherited thromboembolic disease, laboratory testing for the most common abnormalities (antiphospholipid antibody syndrome, is factor V Leiden, prothrombin G20210A mutation, homocysteine abnormality) usually performed first. If these tests are negative, additional testing for less common deficiency states (fibrinolytic abnormalities, plasminogen deficiency) might be considered. Most deficient individuals presenting with thrombosis are managed acutely with heparin therapy, followed by long-term oral anticoagulant therapy. Commercially prepared concentrates are available for use post-surgically and during parturition in AT III deficient individuals. Protein C concentrates are available on a compassionate use basis (Martinelli ,2003).

1.2.6.1. Molecular Diagnosis.

Thrombophilia can best be defined as a disorder of coagulation that contributes to a predisposition towards thrombosis. Although the term thrombophilia has been used to describe arterial thrombosis, its most common usage has been in reference to venous thromboembolism (VTE). Thrombophilia can be a consequence of both acquired and inherited or genetic causes. Acquired causes include conditions such as surgery, cancer, and prolonged immobilization, while genetic causes have been linked to the inherited deficiencies of anti thrombin, protein C, and protein S (Zhou *et al*., 2005).

The identification of the genetic basis of these inherited causes of thrombophilia ushered in a new way of thinking about thrombosis and the importance of its genetic component. Interest in the genetic basis of VTE was accelerated with the subsequent discovery of factor V Leiden, prothrombin G20210A, and MTHFR C677T. These single nucleotide polymorphisms (SNPs) and other genetic variants associated with VTE have become fixtures in the molecular diagnosis of inherited thrombophilia. Because of the large volume of current and anticipated future genetic testing, there has been a push to develop many different genotyping methods which are now used in both clinical and research settings. The identification of new genetic variants that may either directly or indirectly affect coagulation or the anticoagulant pathway, may greatly advance the understanding and clinical management of thrombophilia (Clin , 2005).

To make genetic diagnosis and pedigree analysis for patients with recurrent venous thromboembolism due to inherited deficiency of protein C (PC), protein S (PS). The routine coagulation tests including activated partial thromboplastin time (APTT) , prothrombin time (PT) and thrombin time (TT) were performed , chromogenic substrate assay was used to detect the activities of Protein C (PC), total Protein S (PS) and antithrombin (Coppola *et al*., 2010).

All exons and their flanks of PC and PS gene were amplified by polymerase chain reaction (PCR). The PCR products were sequenced directly and blastered to normal sequence of corresponding anti-coagulant protein to find the gene mutations (Castoldi *et al*.,1998).

Totally nine probands with DVT or PE were enrolled, their peripheral blood and medical histories collected after informed consents. Proband 1, 2 and 3 were with combined deficiency of PC and PS, while proband 4 was with PC deficiency.

Sequencing of PC gene showed there were polymorphism sites G4880A, C4867T and A5054T in promoter region for all four probands with PC deficiency. PC:C and PS:C for proband 1 was 48% and 26.3%, respectively. PC gene sequencing showed that there was a heterozygous mutation A6578T in exon2 region, resulting in Thr18Ser. Sequencing of PS gene showed there was G68395T heterozygous mutation in exon4 region, leading to Arg90Leu. PC:C and PS:C of proband 2 was 27% and 22.9%, respectively (Asselta *et al*.,2006).

Heterozygous mutations of G68428A and C68430T in exon4 region of PS gene were found, leading to Arg100His and Gln101Stop, respectively. Proband 3 was with PC deficiency and PS deficiency, PC and PS were 58% and 57.3%. Heterozygous AGA12702-12704 or AGA12705-12707 deletion mutation was found in Exon2 of PC gene resulting in Arg192del or Arg193del, and heterozygous missense mutation A15240G in Exon9 resulting in His370Arg. Heterozygous mutation G68395T and G825512C was found in Exon4 and Exon9 region of PS gene, respectively, resulting in Arg90Leu and Ser321Thr. Proband 4 was with PC deficiency, PC:C50% (Banavali *et al*., 2013).

There was no other mutation detected except for polymorphism sites in promoter region. Proband 5 was with PS deficiency, PS:C 38.8%. Heterozygous mutation G68395T in exon4 region was detected, leading to Arg90Leu. Homozygous

mutation C102102T was found in Exon14 region,leading to Gla616Val. Proband 6 was with PS deficiency, PS:C 35.2% (Stanley *et al*.,2007).

Heterozygous mutations G68395T andC68430T in Exon4 were found, leading to Arg90Leu and Gln101Stop, respectively. Proband 7 was with PS deficiency, PS:C 43.7%.Homozygous mutation C102102T in exon14 region was detected, resulting in Gla616Val. Proband 8 was with PS deficiency, PS:C43.6%. Heterozygous mutation G68395T and C68430T in exon4 region was found, leading to Arg90Leu and Gln101Stop. Proband 9 was with PS deficiency, PS:C 7.7% (Altenberg , 2011).

Two of his family members were also with PS deficiency (II2，III2） with heterozygous mutation G68395T in Exon4 region（II1，II2，III1，III2），leading to Arg90Leu. Conclusions: Polymorphisms of G4880A, C4867T and A5054T in promoter region, missense mutation A6578T, A15240G, AGA12702-12704 or 12705-12707 deletion mutation in PC gene，missense mutation G68395T, G68428A, C86066T, G82512C, C102102T, and nonsense mutation C68430T in PS gene might be the cause of reduced activities of corresponding anticoagulant proteins. All these mutations, except for C86066T in PS gene .(Rong *et al*., 2014)

1.2.7. The F5 gene.

F5 gene provides instructions for making a protein called coagulation factor V. Coagulation factors are a group of related proteins that make up the coagulation system, a series of chemical reactions that form blood clots. After an injury, clots seal off blood vessels to stop bleeding and trigger blood vessel repair（Asselta *et al* .,2006).

The factor V protein is made primarily by cells in the liver. The protein circulates in the bloodstream in an inactive form until the coagulation system is

activated by an injury that damages blood vessels. When coagulation factor V is activated, it interacts with coagulation factor X. The active forms of these two coagulation factors (written as factor Va and factor Xa, respectively) form a complex that converts an important coagulation protein called prothrombin to its active form, thrombin. Thrombin then converts a protein called fibrinogen into fibrin, which is the material that forms the clot (Asselta and Peyvandi , 2009) .

Coagulation factor V has another role in regulating the coagulation system through its interaction with activated protein C (APC). APC normally inactivates coagulation factor V by cutting (cleaving) it at specific sites. this inactivation slows down the clotting process and prevents clots from growing too large. when coagulation factor V is cleaved at a particular site (protein position 506), it can work with APC to inactivate factor VIIIa, which is another protein that is essential for normal blood clotting (Brugge *et al*., 2005).

Factor V deficiency caused by mutations in the F5 gene at least 100 mutations in the F5 gene have been found to cause a rare bleeding disorder called factor V deficiency. These mutations prevent the production of functional coagulation factor V or significantly reduce the amount of the protein in the bloodstream. people with this condition typically have less than 10 percent of normal levels of coagulation factor V in their blood; the most severely affected individuals have less than 1 percent. A reduced amount of functional factor V prevents blood from clotting normally, causing episodes of abnormal bleeding that can be severe. factor V deficiency results from mutations in both copies of the F5 gene, although some people with a mutation in a single copy of the gene have mild bleeding problems (Castoldi and Rosing , 2004).

Factor V Leiden thrombophilia - caused by mutations in the F5 gene. factor V Leiden is the name of a specific mutation in the F5 gene. this mutation changes a single protein building block (amino acid) in the factor V protein. Specifically, it

replaces the amino acid arginine with the amino acid glutamine at protein position 506 (written as Arg506Gln or R506Q). Because position 506 is one of the sites where APC normally cleaves coagulation factor V, the factor V Leiden mutation slows the rate at which APC inactivates this factor. As a result, both the activated form of coagulation factor V and coagulation factor VIIIa persist longer in circulation, increasing the risk of developing an abnormal blood clot. This tendency to form abnormal clots that can block blood vessels is known as thrombophilia (Colak *et al*., 2006).

The presence of the factor V Leiden mutation in one or both copies of the F5 gene can cause thrombophilia; two copies of the mutation lead to a higher risk of developing abnormal blood clots than a single copy of the mutation. other disorders- increased risk from variations of the F5 gene (Daksis *et al*.,2007) .

Some people have the factor V Leiden mutation (Arg506Gln) in one copy of the F5 gene and a mutation associated with factor V deficiency in the other copy of the gene in each cell. The factor V Leiden mutation results in the production of an abnormal coagulation factor V protein that is resistant to inactivation by APC, while the other mutation prevents the production of any coagulation factor V protein. This combination of mutations is associated with an increased risk of abnormal blood clots similar to the risk associated with having two copies of the factor V Leiden mutation (Rosendaal *et al*., 2009).

The factor V Leiden mutation is involved in some cases of a condition known as Budd-Chiari syndrome. This condition is characterized by a blockage of blood flow from the liver, which can be caused by a blood clot. People with thrombophilia, including that caused by the factor V Leiden mutation, have an increased risk of developing Budd-Chiari syndrome as shown in figure (1-5) (Hector , 2005). Signs and symptoms of the syndrome include pain in the

abdomen, an abnormally large liver (hepatomegaly), and accumulation of fluid in the lining of the abdomen (Janssen *et al*., 2000).

Cytogenetic Location: 1q23 Molecular Location on chromosome 1: base pairs 169,511,953 to 169,586,530. More precisely, the F5 gene is located from base pair 169,511,953 to base pair 169,586,530 on chromosome 1.

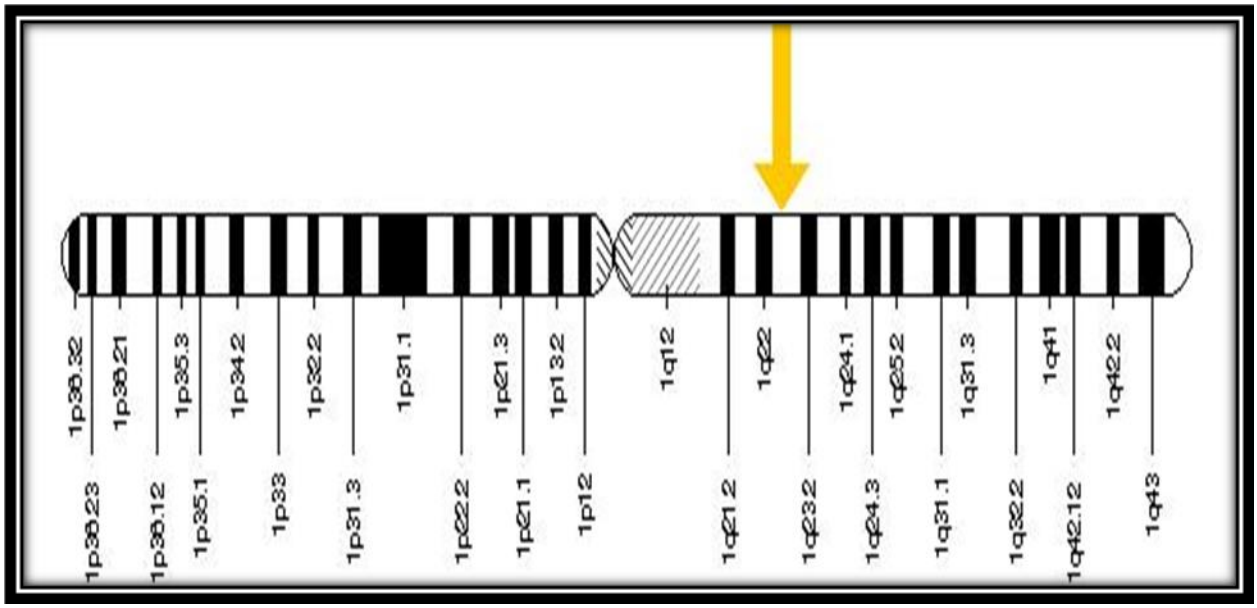


Figure (1-5): The F5 gene is located on the long (q) arm of chromosome 1 at position 23.

1.2.8.Treatment and prevention of thromboembolic disease.

Despite the efficacy of the natural antithrombotic system, some patients require therapeutic agents to prevent over activation of the clotting system. Aspirin, heparin and Coumadin have been available for several decades, but each has limitations (Davidson *et al*., 2003). A wide variety of new drugs are being tested, some have recently received FDA approval show table (1-1) (Bates and Weitz , 2003).

Table (1-1):Properties of Major Anticoagulant Drugs.

Drug	Most Important Applications	Route of Administration	Laboratory Monitoring	Adverse Effects and Precautions
Coumadin (Warfarin)	Thrombosis prophylaxis in outpatients	Oral	Prothrombin time, INR	Bleeding with overdose, skin necrosis and gangrene
Unfractionated heparin	Thrombosis prophylaxis in hospitalized patients	Intravenous Subcutaneous	aPTT, anti-factor Xa assay	Bleeding, thrombocytopenia, acute systemic reaction, skin necrosis, resistance to heparin effect, osteoporosis (long-term use)
Low Molecular Weight Heparins			Anti-factor Xa assay. Usually indicated for only for patients with renal insufficiency or obesity.	Bleeding, thrombocytopenia, osteoporosis (long-term use)
Ardeparin	Total knee replacement	Subcutaneous		
Dalteparin	High-risk abdominal surgery	Subcutaneous		
Enoxparin	Knee or hip replacement, abdominal surgery, DVT treatment, unstable angina, Non-Q wave MIs	Subcutaneous		
Tinzaparin	Treatment of DVT	Subcutaneous		
Direct thrombin inhibitors				
Lepirudin	Treatment of HIT		aPTT, ACT	Contraindicated in renal disease
Argatroban	Treatment of HIT		aPTT, ACT	Contraindicated in hepatic disease
Bivalirudin	Treatment of HIT		aPTT, ACT, thrombin time	Contraindicated in renal disease
Anti-platelet agents				
Aspirin	Thrombosis prophylaxis	Oral	Usually not required. PLT function assays if needed.	Bleeding, GI hemorrhage
Clopidogrel, ticlopidine	Acute coronary syndrome, Coronary intervention, stents	Oral		Not in pregnancy or hepatic disease, neutropenia (ticlopidine only)
GP IIb/IIIa blockers	Same as clopidogrel	Intravenous		Thrombocytopenia, bleeding
Dipyridamole	Strokes	Oral		Headache, GI upset, dizziness
Factor Xa inhibitors				
Fondaparinux	Thrombosis prophylaxis in hip fracture, hip replacement, and knee replacement	Subcutaneous	Usually not required.	Bleeding

1.2.8.1. Aspirin.

Low-dose aspirin (80 mg) is the most commonly used drug for preventing thrombosis, particularly coronary thrombosis in patients with atherosclerosis. Aspirin works by inhibiting an enzyme, cyclooxygenase-1, that is present in platelets and the endothelial cell. A single dose of aspirin works for the life of the platelet (about a week). However, since platelets are continuously produced, aspirin must be taken daily (Chang , 2004).

1.2.9.2. Warfarin (Coumadin).

Crystalline warfarin sodium (Coumadin) is the most widely used oral anticoagulant (Horton and Bush , 1999 ; and Krishnan *et al*., 2003).Warfarin interferes with the synthesis of the vitamin-K dependent procoagulants (factors II, VII, IX, X) in the liver by inhibiting the reduction of oxidized vitamin K. Since functional circulating clotting factors are not affected by warfarin, a week or more of oral anticoagulation therapy is required to achieve an optimal therapeutic effect.Warfarin is a safe agent for the prophylaxis of thrombosis if the correct dosage is given and the patient is carefully monitored (Gage *et al*., 2000; and Schafer , 2003). However, serious bleeding complications can occur with excessive anticoagulation, while thromboembolic complications are a risk with inadequate coagulation. For this reason, accurate laboratory measurements of the prothrombin time (PT) are critical in the management of patients receiving oral anticoagulation (Riley *et al*., 2010).

1.2.9.3. Heparin.

Heparin is the other major anticoagulant used for therapeutic purposes. It is a negatively charged, highly sulfated mucopolysaccharide with a molecular weight between 6,000 and 25,000 Dalton's. It is not absorbed from the gastrointestinal tract and must be given by injection into the veins (intravenous) or under the skin

(subcutaneous). A single intravenous dose has a half-life of approximately 60 minutes. Heparin exerts its potent anticoagulant effect by activating a natural anticoagulant, antithrombin III (Morris , 2003 ; and Valenstein *et al*., 2004).Recent drugs derived from heparin and termed "low molecular weight heparin" act in the same way but have fewer side effects and require less frequent . A particularly serious complication of heparin anticoagulation is thrombocytopenia induced by an immune reaction against complexes of heparin and platelet factor (Spinler and Dager , 2003).

1.2.10. Etiology.

Several causes can be involved in the pathogenesis of PVT and, frequently, more than one coexist. A simple classification distinguishes between local (70%) and systemic (30%) risk factors as shown in Table (1-2) and (1-3) (Wang et al., 2005). Inflammatory abdominal foci (such as appendicitis, diverticulitis, inflammatory bowel diseases, pancreatitis, cholecystitis, hepatic abscesses, and cholangitis), liver cirrhosis or tumors, represent the most common local thrombotic risk factors (Condat and Valla, 2006) **.**

Malignancies, frequently of hepatic or pancreatic origin, are responsible for 21%-24% of overall cases of PVT .Direct vascular invasion, compression by tumor mass, or a hypercoagulable state are the mechanisms involved in neoplastic PVT development; hormonal factors might also play a role in this process, especially in men (Kocher and Himmelmann , 2005).

PVT is common in patients affected by liver cirrhosis, with a risk related to the severity of the disease; the prevalence ranges from 1%, at the earlier stages, to 30% in candidates for liver transplantation (Chawla *et al*., 2009). Moreover, in patients

with a hepatocellular carcinoma, the incidence of PVT rises to 10%-40% (Hoekstra and Janssen ,2009).

Table (1-2): Most frequent local risk factors for PVT .

Most frequent local risk factors for PVT
Local risk factors for PVT (70%)
Cancer
Any abdominal organ
Focal inflammatory lesions
Neonatal omphalitis, ombilical vein catheterization
Diverticulitis, appendicitis
Pancreatitis
Duodenal ulcer
Cholecystitis
Tuberculous lymphadenitis
Crohn's disease, ulcerative colitis
Cytomegalovirus hepatitis
Injury to the portal venous system
Splenectomy
Colectomy, gastrectomy
Cholecystectomy
Liver transplantation
Abdominal trauma
Surgical portosystemic shunting, TIPS
Iatrogenic (fine needle aspiration of abdominal masses *etc*.)
Cirrhosis
Preserved liver function with precipitating factors (splenectomy, surgical portosystemic shunting, TIPS dysfunction, thrombophilia)
Advanced disease in the absence of obvious precipitating factors

Table (1-3): Most frequent systemic risk factors for PVT.

Most frequent systemic risk factors for PVT
Systemic risk factors for PVT (30%)
Inherited
Factor V Leiden mutation
Factor II (prothrombin) mutation
Protein C deficiency
Protein S deficiency
Antithrombin deficiency
Acquired
Myeloproliferative disorder
Antiphospholipid syndrome
Paroxysmal nocturnal hemoglobinuria
Oral contraceptives
Pregnancy or puerperium
Hyperhomocysteinemia
Malignancy

Other less common PVT local causes are adenopathy, systemic inflammatory response syndrome, and surgical traumas to the portal venous system, such as portosystemic shunting, splenectomy, liver transplantation, ablative therapy for HCC, and fine needle aspiration of abdominal masses (Bayraktar and Harmanci 2006).

On the other hand, myeloproliferative disorders and prothrombotic conditions belong to the group of systemic risk factors, with a prevalence of about 40% and 60%, respectively as shown in Table (1- 4) (Arcadipane *et al*., 2008).

Table (1-4): Prevalence of thrombotic risk factors in series of routinely investigated, consecutive adult patients with non tumorous and non cirrhotic, acute or chronic, PVT.

Risk factor	PVT patients (%)
Myeloproliferative disorders	**30-40**
Atypical	**14**
Classical	**17**
Antithrombin deficiency	**0-26**
Protein C deficiency	**0-26**
Protein S deficiency	**2-30**
Factor V Leiden mutation	**6-32**
Prothrombine mutation	**14-40**
TT677 methylene tetrahydrofolate reductase (MTHFR) genotype	**11-50**
Antiphospholipid syndrome	**6-19**
Hyperomocisteinemia	**12-22**
Recent pregnancy	**6-40**
Recent oral contraceptive use	**12**

Factor V Leiden mutation is the most common thrombophilia predisposing to Dutta *et al*., 2008).(PVT, followed by protein C (PC) deficiency

The role of protein S (PS) and antithrombin III (AT) deficiency in PVT etiology has not yet been confirmed, and it is difficult to evaluate the influence of anticoagulation therapy on the impairment in liver function. Indeed, in cirrhotic patients it is hard to distinguish between congenital and acquired deficiencies of natural coagulants and their role in PVT pathogenesis, because if liver function is impaired, levels of coagulation inhibitors, as well as those of pro-coagulation factors, are often decreased (Amitrano *et al*., 2000).

A clinical study conducted on eleven children with PVT reported a significant improvement in PC, PS, factors II, V, and VII levels and prothrombin time after surgical correction with a Rex Shunt (mesenteric-left portal vein bypass).

In contrast, a distal spleno-renal shunt or an H-type meso-caval shunt, in the same condition, did not seem to be equally effective, probably due to insufficient residual portal vein flow and the consequent impairment in liver synthetic function (Mack *et al*.,2003).

However, the relatively low prevalence of genetic, in respect to acquired, thrombophilic disorders, might represent a potential diagnostic matter in PVT patients, and should be considered carefully in clinical practice. To overcome this problem, an accurate genetic study of the patient and, eventually, his/her family (first degree relatives) might be useful in strongly suggestive cases. Unfortunately, in practice, this policy is not applicable without difficulty. A simple method to screen the deficiency of natural anticoagulants in patients with liver disease comprises the ratio of PS or PC or AT to [(factor II + factor X)/2. If the result is less than 70%, a genetic deficiency has to be suspected and investigated (Bayraktar and Harmanci , 2006).

Among the other thrombophilic disorders, a prothrombin gene mutation seems to be frequent among cirrhotics with PVT. However, in the general population, its role in PVT development seems less clear, as it is considered a weak prothrombotic risk factor. Moreover, a homozygous methylene tetrahydrofolate reductase (MTHFR) gene mutation might be associated with PVT development alone or, if heterozygote, in the presence of other cofactors. Reported a strong correlation between the prothrombin A20210 mutation or the homozygous MTHFR C677-T genotype and PVT in cirrhotic patients without evidence of liver cancer (Baxter *et al*.,2005).

Furthermore, the presence of anticardiolipin antibodies is quite frequent in patients with chronic liver disease; a transient positivity is often reported after infections, suggesting a relationship between microorganisms (Bacteroides species) and thrombotic events, such as PVT. In contrast, other studies consider

anticardiolipin antibodies simply as an epiphenomenon of liver damage (Oksüzoglu *et al*.,2003).

Finally, the role of oral contraceptives, steroids, and pregnancy is still less clear In about 22%-48% of patients, PVT is a manifestation of a myeloproliferative disease (MPD).An intra-abdominal vascular thrombosis is often the sole presenting symptom and an overt MPD might successively develop in 51% of cases. In the Western Countries, 58% of idiopathic PVTs are associated with a latent MPD. The principal diagnostic criteria are usually incompletely met in these patients, probably because of the atypical manifestation of the disease. The 1849G→T point mutation in the gene encoding tyrosine-protein kinase JAK2, is a specific and easily detectable marker for MPDs, which can often be useful for a rapid diagnose in PVT patients. Recent studies reported the presence of a JAK2 mutation in about 17%-35% of patients with PVT, but further studies are needed to confirm these data (Colaizzo *et al*.,2007).

Occasionally, it is not possible to recognize any overt cause of PVT; generally, the clinical course is favorable for these patients, with a low incidence of complications. However, at present, "idiopathic PVT" is less frequent, thanks to the amelioration in diagnostics and to a more scrupulous attention to patients' clinical history. In conclusion, it is reasonable to routinely investigate the most common prothrombotic disorders and exclude a local trigger, to provide a correct management of PVT and its original cause. However, the mechanism of PVT development is complex and multifactorial, and is not always attributable to a single risk factor. In the presence of sporadic local or systemic promoting events, an underlying intrinsic predisposition might be the access key to thrombosis development (Pan,2009).

CHAPTER TWO

Materials and Methods

2. Materials and Methods.

2.1. Materials.

2.1.1. Apparatus.

Various apparatuseswere used in this study as listed in Table (2-1).

Apparatuses used .:Table (2-1)

Apparatus	Company / country
Analytical balance	FC-400 / China
Autoclave	HIRAYAMA
Bench centrifuge	Uni-Media / Korea
Electrophoresis equipment	JUNY1-JY200C
Gel –documentation	Bio-Red / U.S.A
Heater – magnetic stirrer	Stuart / England
Microcentrifuge	MIKRO120 – Hettich
Microwave	LG / Korea
Nanodrop spectrophotometer	Thermo / USA
Oven	Sanyo / Japan
GradientPCR thermal cycler	Techne / U.K.
Conventional PCR	Techne / U.K.
pH meter	Martini / Japan
Sensitive balance	Mettler / Switzerland
U.V. Transilliuminator	Flowgen / U.K.

Vortex	Scientific industries / U.S.A.
Water bath	Memmert / Germany

2.1.2. Chemicals.

Various chemicals were used in the study as listed inTable (2-2).

chemicals.:Table (2-2)

Chemicals	**Company / country**
Absolute ethanol	Phamacia / sweeden
Agaros	Biobasic / Canada
DNA ladder	Promega / USA
Deionized water	Bioneer / korea
Ethediumbromid	Biobasic / Canada
Loading dye	Biobasic / Canada
Green master mix	Promega /USA
Primers	Bioneer / Korea
Tris Borate EDTA (TBE)	Biobasic / Canada
Monoclonal anti-cTnI antibody gold (mouse)	Medpointmedikal/Turkey
anti-cTnI(mouse)	Medpointmedikal /Turkey
anti- mouse IgG antibodies(goat)	Medpointmedikal /Turkey

2.1.3. Kit.

The kits used in the study are listed in table (2-3).

the kit used.:Table (2-3)

Kit	Company	Country
Mini DNA Extraction Prep System	Promega	U.S.A

2.1.4. Primers.

The sequences of the primers used in table (2-4).

Sequences of the primers used.:Table (2-4)

No	Name	Oligonucleotides	Tm	GC%	Product size	Sequence (5'-3')	Ref.
1-	-F٣Fve	Forward primer	٥٣.٤٩	٤٣.٤٨	228bp	GATGACCCTGAATACAGACATAG	(Van *etal*.,2001)
	-٣FveR	Reverse primer	٥٢.٢٦	٣٧.٥٠	-	GATGCTGGTATTAAAGACTTAGAC	(Van *et al*.,2001)
2-	Fve4-F	Forward primer	٥٤.٣٦	٥٢.٣٨	310bp	ACTGCCCACATGTCTTGATGG	(Van *et al*.,2001)
	Fve4-	Revers	٥٤.٨	٥٠.٠	-	TGACAGACT	(Van *et*

	R	e primer	٤	٠		CCTGACCAT TCC	*al*.,2001)
3-	Fve6-F	Forwa rd primer	٥٥.٢ ٧	٤٧.٨ ٣	547b p	GCCTAATCC TTTAGCAAT CCCTG	(Van *et al*.,2001)
	Fve6-R	Revers e primer	٥٥.٢ ٧	٤٧.٨ ٣	-	CATTGAGAA GCAAGACTG TCAGG	(Van *et al*.,2001)
4-	Fve7-F	Forwa rd primer	٥٢.٧ ٦	٣٦.٠ ٠	241b p	GAGTTATTTC ATTGTCTTTC TGTCC	(Van *et al*.,2001)
	Fve7-R	Revers e primer	٥١.٠ ٩	٥٢.٦ ٣	-	GTCTTGAAC CTTTGCCCA G	(Van *et al*.,2001)
5-	Fve8-F	Forwa rd primer	٥٠.٤ ٥	٤٢.٨ ٦	306b p	GCAGAATGT TTAAGCACA AGG	(Van *et al*.,2001)
	Fve8-R	Revers e primer	٥٢.٧ ٦	٣٦.٠ ٠	-	CTATGTAATT TCTCCCATG ATTCTG	(Van *et al*.,2001)
6-	Fve12-F	Forwa rd	47.3 8	31.8 2	286b p	CATAGACTT GGAATTTTA	(Van *et al*.,2001)

		primer				ACAG	
	Fve12-R	Reverse primer	54.36	52.38	-	CAAGCTTCCTCTGTGAGTGTC	(Van *et al*.,2001)
7-	Fve13a-F	Forward primer	51.78	50.00	260bp	GTCTTTTCCCAGACTTCCAG	(Van *et al*.,2001)
	Fve13a-R	Reverse primer	51.11	40.91	-	TGTTCTGGTAATCATAGTCAGC	(Van *et al*.,2001)
8-	Fve13c-F	Forward primer	48.93	47.37	317bp	ATAGTGGGCCTCAGTAAAG	(Van *et al*.,2001)
	Fve13c-R	Reverse primer	41.21	33.33	-	TTTTTTCAGCAGTAATGG	(Van *et al*.,2001)
9-	Fve15-F	Forward primer	53.83	55.00	600bp	GGCCATATCTCACAGGATGG	(Van *et al*.,2001)
	Fve15-R	Reverse primer	54.36	52.38	-	GTCATCTGAAGAGCTGCATGG	(Van *et al*.,2001)

10-	Fve16-F	Forward primer	49.73	45.00	333bp	TCCTGAGAAAGAGGCAATAC	(Van *et al*.,2001)
	Fve16-R	Reverse primer	47.68	40.00	-	TCTTGTGAATATCTAAGGGC	(Van *et al*.,2001)
11-	Fve25-F	Forward primer	48.14	30.43	390bp	AGCCATTTATGTTGTCATTAAAG	(Van *et al*.,2001)
	Fve25-R	Reverse primer	48.84	29.17	-	TAATAGCCATTATCTTACTTACTG	(Van *et al*.,2001)

2.2. Study subjects.

The study included 40 patients suffering fromthrombosis during the period from November 2014 to January2015AL Kadhimiya Hospital Teaching and Yarmouk Hospital Teaching . The apparently healthy 10 person individuals from college of science / Al Nahrain University were selected to represented the control group. The years and the maen ages of the control ٧٦maen ages of the patients were 34 - were 25 - 40 years. Informed consents from patient as well as control were taken which included age and family history of thrombosis.

2.3. Blood samples collection.

Five ml of blood was collected kept in EDTA tube and preserved -20 °C until be used .

2.4. DNA Extraction ‘Mini Prep System kit.

The Reliaprep Blood genome. Isa DNA MiniPrep SystemfromPromega USA, ready to use that contained the following Component:

- Cell Lysis(buffer)
- Proteinase K Solution (PK)
- Binding buffer
- Column Wash Solution
- Collection Tubes
- Nuclease Free Water

2.5. Green Master Mix.

Go Taq® Green Master Mix is a premixed, ready to use solution containing bacterially derived Taq DNA polymerase, dNTPs, Mgcl 2 and reaction buffers at optimal concentration for effective amplification of DNA templates by PCR.

2.6. DNA ladder (100 bp).

DNA ladder 100- 3000bp containing 13 fragments supplied in storage buffer 10mM Tris-HCL pH (7.5), 0.1 mM EDTA.

2.7. Methods.

١2.7. Sterilization methods.

- Autoclaving: Buffers and solutions were sterilized by pressure vessel (autoclave) at 121^0C and 15 bar for 15 minutes.
- Dry heat: A laboratory oven was used for glassware sterilization. Glassware was placed in the oven at 200^0C for 2 hours.

. Detection in thrombosis laboratorybyTroponin test(TNI)(Jaffe *et al*., ٢2.7. 2000).

.1.Test principle .٢2.7.

The test employs a monoclonal anti-cTnIantibody gold conjugate (mouse) in the mobile phase, monoclonal anti-cTnI antibodies (mouse), fixed in the test line, and polyclonal anti-mouse IgG antibodies (goat) in the control line.

As the sample flows through the absorbent pad, human troponin I is boundby the anti-cTnI antibodies in the test line and produces a red-violet test line (T). Excess conjugate reacts in the control line with the anti-mouse IgG antibodies, forming a second red-violet demonstrate the correct function of the reagents.

TEST Monoclonal anti-cTnI antibody gold (mouse),anti-cTnI(mouse) and anti-mouse IgG antibodies(goat).

PIP 20 disposable dropper pipettes.

.2. Test procedure.٢2.7.

1. Allow specimen and **TEST** to reack room temperature (15-30^0C) prior to testing.

2. Remove **TEST** from its pouch and use it as possible.

3. lablel**TEST** for patient identification.

4. By holding **PIP** vertically, dispense 3 free falling drops (approximately 100µl) of sample into the round sample window at the lower end of **TEST**. Avoid bubbles in the sample window when adding liquids. Remaining residues of sample in the sample windo at the end of the incubation period can be neglected .

5. Read results at 15 minutes at a well lit place. To avoid incorrect readings or invalid results, do not read after 15 minutes.

.3.Interpertation of results.٢2.7.

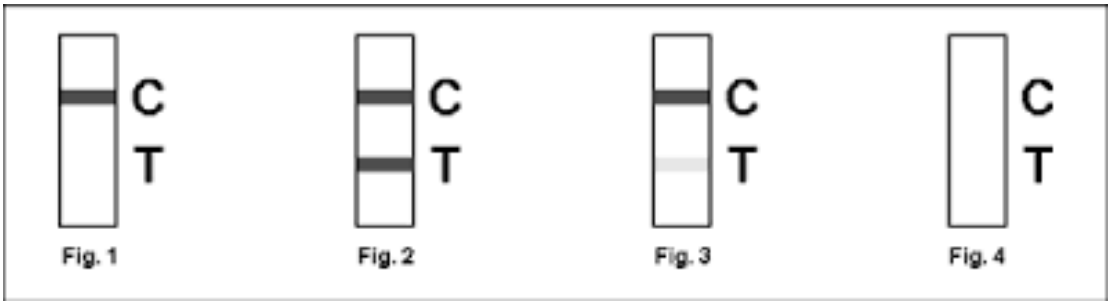


Figure (2-1): Interpertation of results the Troponin test.

Negative (fig.1).

Only one red-violet control line (C) appears in the upper part of the rectangular result window showing that the test has been carried out correctly.

Positive (fig.2and 3).

A second red-violet test line (T) appearing in the lower part of the rectangular result window, indicates a positive result for cardiac tpoponin I in the sample.

Even a weak line indicates apositive result (fig .3).

Different intensities between test (T) and control (C) lines may occur but are irrelevant for the Interpertation of the result.

Invalid (fig .4).

If no control line appears, even if a test line is visible, the test has to be reeated with a fresh **TEST**.

. DNA extraction from blood samples.٣2.7.

.1.Procedure.٣2.7.

The Extraction was briefly carried out as follow:

1. The blood Sample was mixed thoroughly for at least 10 minutes in a rotisserie at room temperature.
2. A liquate of 20 µl of Proteinase K Solution was added into Micro centrifuge tube.
3. A liquate of 200µl of blood was added to the Proteinase K Solution and mixed briefly.
4. A liquate of 200µl of Cell lysis(blood) buffer was added to the tube and mixed for at least 10 seconds by vortexing ,then incubated at 56^0C for 10 minutes.
5. A liquate of 250 µl of Binding Buffer was added to the tube and mixed for 10 seconds by vortexing.
6. The Sample mixture was transferred to a filter column set and centrifuged at maximum speed for 1 minute.

7. The collection tube containing the supernatant was discarded and replaced by a fresh collection tube.
8. A liquate of 500µlof column wash solution was added to the column and centrifuged for 3minute at maximum speed and thesupernatantwas discarded.
9. A liquate of50 µlof Nuclease Free Water was added to the column and centrifuged for1minute at maximum speed.
10. The column was discarded and the eluted was stored at -20^{0}C.

2.7.٣.2. Measurement of extracting DNA concentration and purity.

Ananodrop spectrophotometer (Thermo / USA) was used to estimate the concentration and purity of the extracted DNA (from blood of patient and control) according to the following procedure.

.2.1. Blanking.٣2.7.

1- After lifting the sample arm, 2 µl of nuclease free water was pipetted on to the measurement pedestal.
2-The sampling arm was laid down and the solution was observed bridging the gap between both optical fibers.
3- When the Blank button was clicked, the apparatus measured the solution with both 1 mm and 0.2 mm path lengths, and then the system recorded both results automatically.
4- When the measurement completed, the sample arm was opened and then the banking buffer was wiped from pedestal using laboratory wipe.

.2.2.Sample measurement.٣2.7.

1- Two µl of the sample was pipetted on to the measurement pedestal while the sample arm is opened.
2- The sampling arm was laid down and the solution was observed bridging the gap between both optical fibers.
3- The button (Measure) was clicked and the measurement result appeared on the screen within 3 sec.
4- When any sample gives purity less than 1.5 and /or concentration less than 10 ng/µl DNA extraction from the blood of that sample re-performed until the desired .purity and concentration were obtained

. PCR protocols.٤2.7.

Extracted DNA from blood samples and healthy was used in PCR for amplification of (Fve3, Fve4, Fve6, Fve7, Fve8, Fve12 ,Fve13a, Fve13c, Fve15,Fve16,Fve25).

PCR amplification...1٤2.7.

For PCR amplification of F5 gene the following PCR protocols was followed:

1. The Go Taq® Master (Promega / USA) Mix was thawed at room temperature. The master mix was mixed by overtaxing then it was spined briefly in a microcentrifuge.
.Component of Taq® Master mix are illustrated in table (2-5)

Table (2-5): component of Taq® Master mix.

No.	Component	concentration
1-	Taqpolymerase	2.5µl
2-	dNTP(dATP, dCTP, dGTP, dTTP)	250µM

3-	Tris – HCL (ph 9.0)	10 mM
4-	KCL	30mM
5-	Mgcl	1.5mM

٢. The reaction mixture was prepared by adding the components listed in table (2-6).

Table (2-6): The optimal values of various steps in the amplification.

Components	Volume	Concentration
Go Taq® Green Master Mix	12.5 µl	1x
Forward primer	0.2 µl	0.2 µM
Reverse primer	0.2 µl	0.2 µM
DNA template	3 µl	> 250 ng
Nuclease free water	9.1 µl	-
Final volume		25µl

3.The mixture was put in microcentrifuge and spinned for better mixing.

٤. After mixing the master mix tubes were transferred to the thermocycler (Techne 32 thermal block / UK) which was previously programmed with the above protocol according to the F5 gene.

.2. Optimal protocol of PCR amplification.ٔ2.7.

For PCR amplification of F5 gene (Fve3, Fve4, Fve6, Fve7, Fve8, Fve12 ,Fve13a, Fve13c, Fve15,Fve16, Fve25)annealing temperature in optimizing thefollowing PCR protocols as followed :

PCR Amplification using Fve3 primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	35cycle	94^0C for 1 minute
Annealing		58^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve4 primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute

Denaturation	35cycle	94^0C for 1 minute
Annealing		60^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve6 primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	35cycle	94^0C for 1 minute
Annealing		58^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve7 primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute

Denaturation	35cycle	94^0C for 1 minute
Annealing		54^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve8primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	30cycle	94^0C for 1 minute
Annealing		54^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve12 primer

Thermal cycler protocol	No. of cycle	Temperature –time

Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	35cycle	94^0C for 1 minute
Annealing		56^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve13a primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	35cycle	94^0C for 1 minute
Annealing		54^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve13c primer

Thermal cycler protocol	No. of cycle	Temperature –time

Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	35cycle	94^0C for 1 minute
Annealing		50^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve15 primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	35cycle	94^0C for 1 minute
Annealing		61^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve16 primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	35cycle	94^0C for 1 minute
Annealing		52^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

PCR Amplification using Fve25 primer

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94^0C for 5 minute
Denaturation	35cycle	94^0C for 1 minute
Annealing		52^0C for 1 minute
Extension		72^0C for 1 minute
Final Extension	1 cycle	72^0 C for 10 min.

. Agarose gel electrophoresis (Sambrook,2002).**^2.**

1. Two percentage of agarose gel was prepared by mixing: 100ml of 1X TBE buffer and 2 gmagarose in a glass bottle. A glass bottle was heated in a magnetic stirrer with heater until the components were dissolved.
2. This solution was cooled to 70^{o}C, 2 µl ethidiumbromides was added from stock solution and mixed thoroughly.
3. The clean glass mold (17X12X4 cm) was set on a horizontal section of the bench. The comb was set in position 0.5-1.0 mm above the surface of the mold so that a complete well was formed when agarose was added.
4. The warm agarose – solution was poured into the mold.
5. After the gel was completely set (20-30 min. at room temperature), the comb was carefully removed and the gel mounted in the electrophoresis tank which contain previously small amounts of 1X TBE buffer.
6. A volume of 600ml of 1X TBE was added to cover the gel in depth about 1mm.
7. A volume of 10 µl of the sample of DNA (PCR product) was added slowly into the slots of the submerged gel using an automatic micropipette.
8. A volume of 5 µl of DNA marker was mixed with 1 µl of loading buffer. The mixture was set slowly into the slots of the submerged gel using an automatic micropipette.
9. The gel was subjected to electrophoresis at 70 volts until the bromophenol blue tracking dye, migrated at least two-thirds of the way down the gel.
10. The gel was examinedby ultraviolet light using a UV transilluminatorthem photographed.

.Measurement of DNA concentration before sequencing.٩2.

Before sequencing DNA concentration of the F5 gene (Fve12 ,Fve13a, Fve13c, Fve16 and Fve25) PCR products from 30 blood samples was measure using nanodrop (techne UK) as in section 2.7.3.2. All products gave concentration more than 100ng/µl which is the least concentration required for DNA to be sequenced.

. DNA sequencing.١٠2.

Polymerase chain reaction products of F5 gene (Fve12 ,Fve13a, Fve13c, Fve16 and Fve25) 30 blood samples were sent toMacrogenCompanyin KoreaforDNA sequencing. The obtained sequences were aligned using NCBI software with normal sequence from NCBI GenBank and examined of the presence of SNPs.

. Statistical analysis.١١2.

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage in this study.

Chapter Three

Results and Discussion

3. Result and discussion.

.3.1. The distribution of the studied group

In this study,fourty samplehave been collected from patient have thrombosis.and used to effect of difference factors in study the age and gender. Chi-square test was results .used to significant compare between percentage in this study(SAS, 2012) are shown in tables (3.1,3.2,3.3,3.4 and 3.5).

Table (3-1):Compare between patient and control in distribution of test of Troponin.

group	Number	No. (%)	
		Ve +	Ve -
Patients	40	40 (100.0)	0 (0.00)
Healthy(Control)	10	0 (0.00)	10 (100.0)
Chi-square value (χ^2)	---	15.00 **	15.00 **
** (P<0.01).			

Table (3-1)test of trponin , Patients (40) positive and healthy (10) negative, the high significant (P<0.01).

Table (3-2): Distribution of patients according to age group.

Age group (year)	Number	Percentage (%)
Less than 50	8	20.00
50-60	18	45.00
More than 60	14	35.00
Total	40	100%

Chi-square value (χ^2)	---	9.041 **
** (P<0.01).		

Table (3-2)the ages less than 50 their percentage 20% from patients, While ages between 50 to 60 their percentage 45% from patients and the ages more than 60 their percentage 35% from patients , the high significant (P<0.01).

Table (3-3): Distribution of patients according to gender.

Gender	Number	Percentage (%)
Male	22	55.00
Female	18	45.00
Total	40	100%
Chi-square value (χ^2)	---	4.529 *
* (P<0.05).		

Table (3-3)the males their percentage 55% from patients, while the females their percentage 45% from patients, the significant (P<0.05).

Table (3-4): Relationship between age group and test of Troponin in patients.

Age group (year)	Number	No. (%)		Chi-square value (χ^2)
		Ve +	Ve -	
Less than 50	8	8 (100.0)	0 (0.00)	15.00 **
50-60	18	18 (100.0)	0 (0.00)	15.00 **
More than 60	14	14 (100.0)	0 (0.00)	15.00 **
** (P<0.01).				

Table (3-4)the ages less than 50 they numbered (8) positive from patients in the test of troponin ,the ages between 50 to 60 they numbered (18) positive from patients in the test of troponin and the ages more than 60 they numbered (14) positive from patients in the test of troponin , the high significant (P<0.01).

Table (3-5): Relationship between gender and test of Troponin in patients.

Gender	Number	No. (%)		Chi-square value (χ^2)
		Ve +	Ve -	
Male	22	22 (100.0)	0 (0.00)	15.00 **
Female	18	18 (100.0)	0 (0.00)	15.00 **
** (P<0.01).				

)The males as number (22) positive from patients in the test of troponin, °Table (3- and the femalesas number (18) positive from patient in the test of troponin .in Chi – squaretest the males were significant on the females and the P value was (P<0.01).

In Azadi hospital in Kirkuk city of Iraq from 2008 to 2009, the percentage of thrombosis with gender, in the Males were more affected with thrombosis (34.2%) than females (13.4%), also with age show that the advanced age groups were more affected the disease (above 65 years) (45%) than early age groups (Wafa and Lamia , 2011).

Thrombosis disease are regarded as the most dangerous disease and their structure which prevent the blood supply to reach the heart that result to sudden failure in circulation, it registered Mortality of 7.6 million deaths caused by the disease in 2005, of all deaths in the world (Gregg *et al*., 2007).

3.2. Molecular detection of thrombosis by PCR technique.

3.2.1. Factor V gene.

The factor Vgene is located on the first chromosome (1q23). It is genomically related to the family of multicopper oxidases, and is homologous to coagulation factor VIII. The gene spans 70 kb, consists of 25 exons, and the

Huang (resulting protein has a relative molecular mass of approximately 330 kDa and Koerper,2008) .

3.2.2. Primers set.

Indentification of nucleotide changes in 3,4,6,7,8,12,13,15,16 and 25 exons performed in all samples with the primers used in the previously in the study.

The first primer set used in this PCR technique(Fve3) the amplifies in intron (2)of F5 gene from NCBI with product size(228bp)which is shown in the figure (3-1).

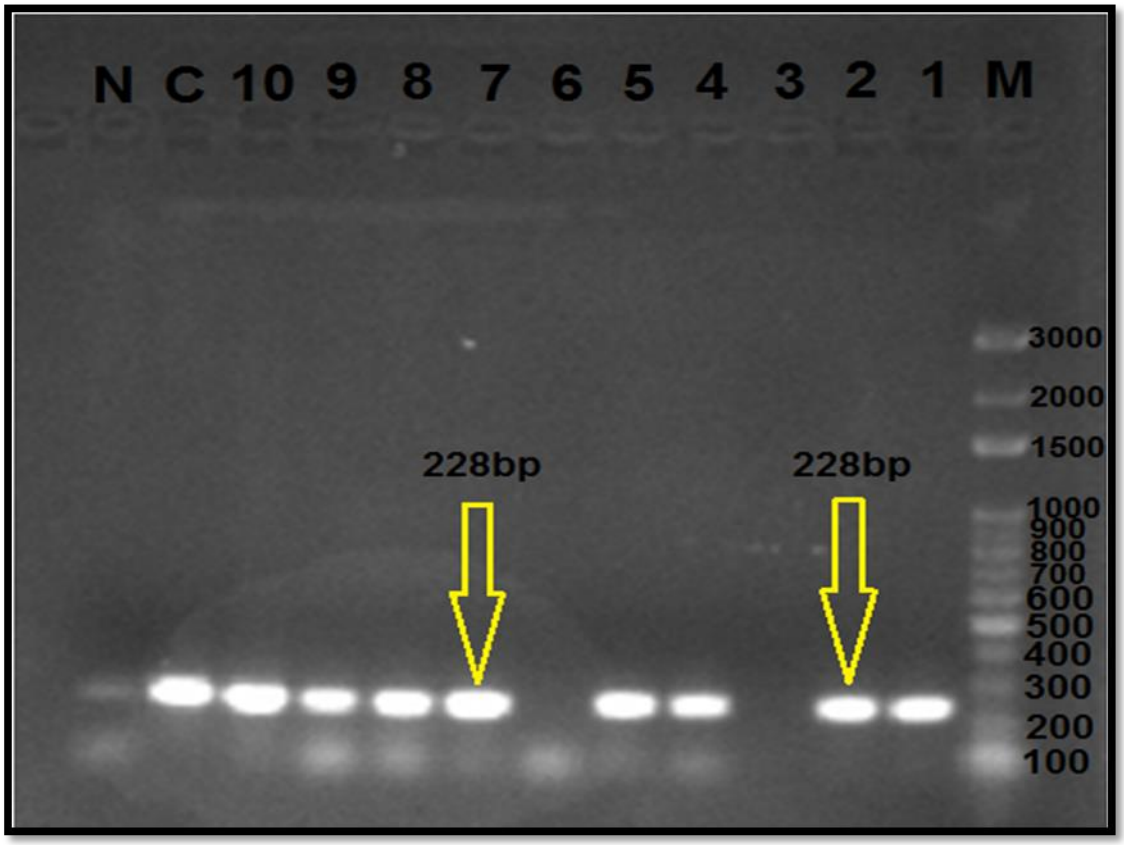
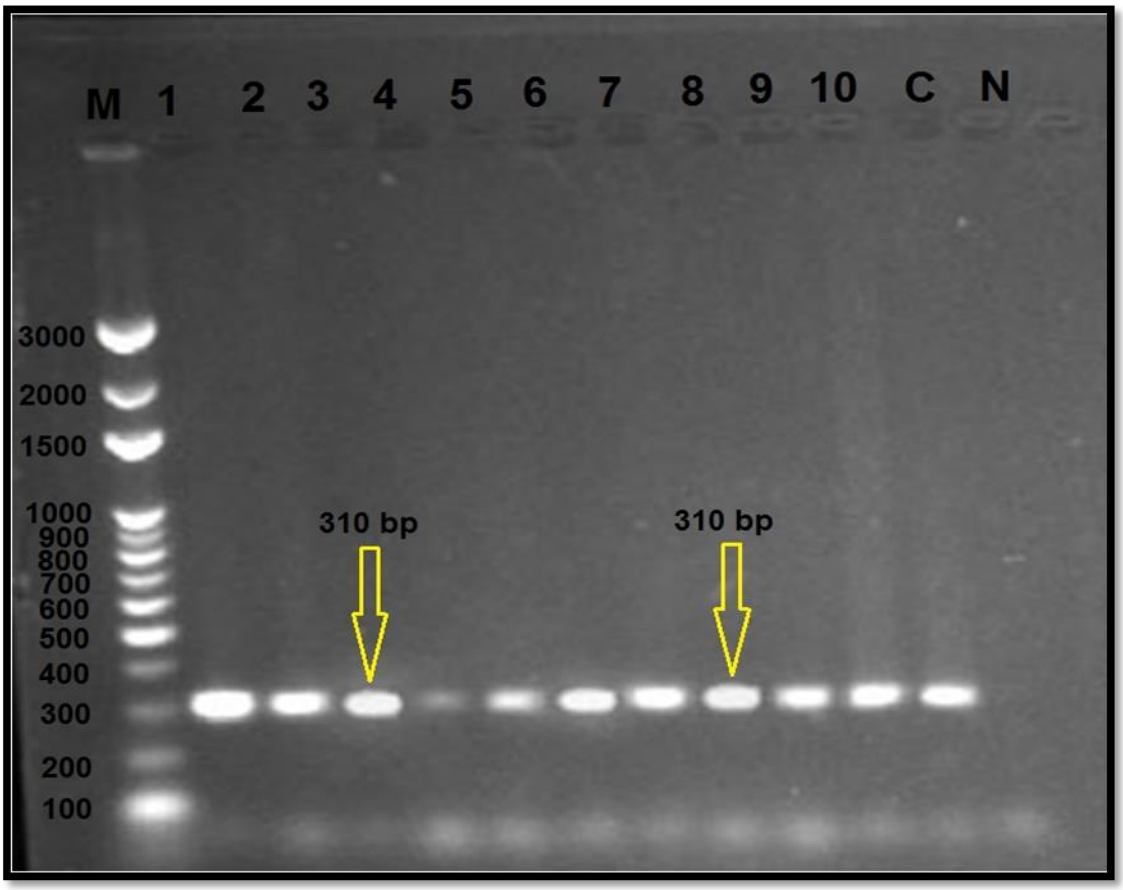


Figure (3-1): PCR product for Fve3 primer, agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltage and then lowered to 70 Volts, 30-minutes). Visualized under U.V light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy) and lineN: from negative control.

) used in this study were previously designed to amplify the F5 gene, ٣Primer (Fve bp). and show ٢٢٨,8,9 and 10 giveband in PCR product (٧,٥,٤show the samples1,2, the sample 3 and 6 did not give any bands during PCR amplification because this primer has no attachment site on the target DNA.

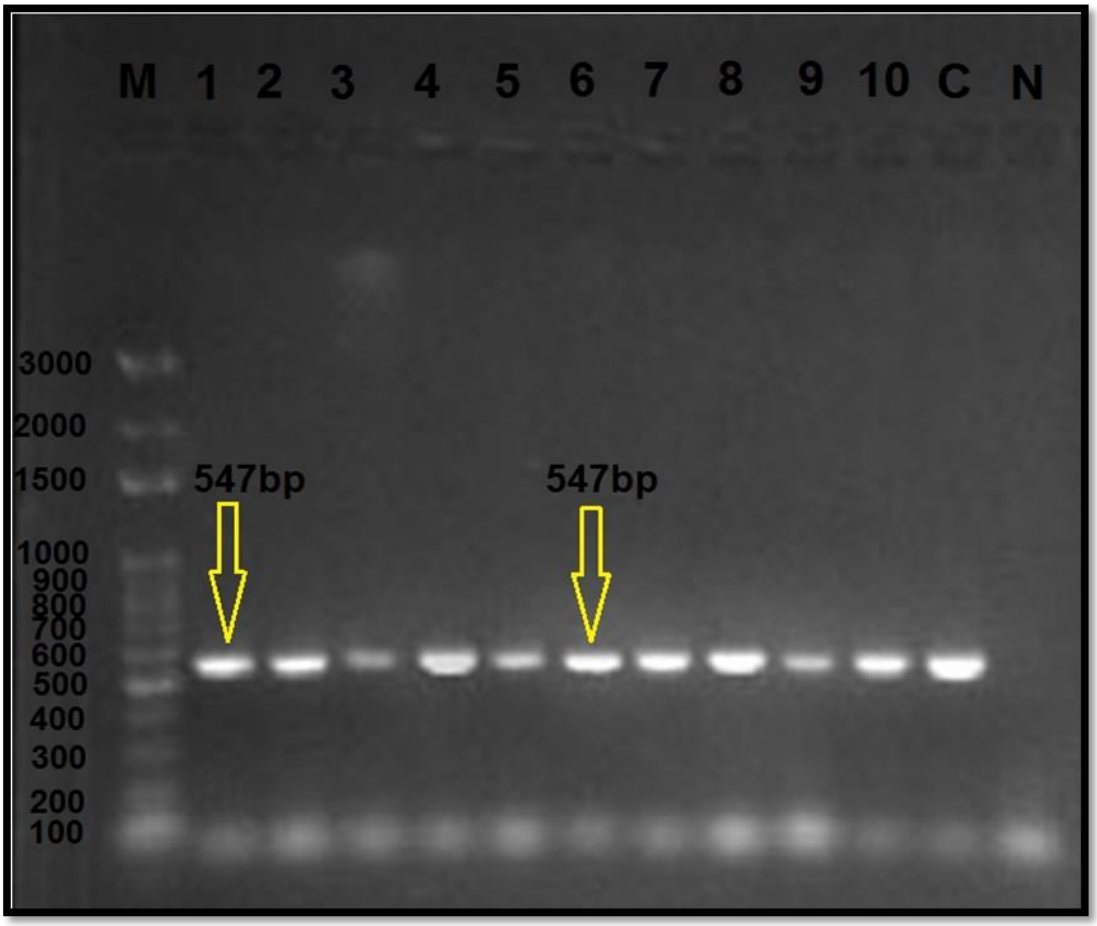
The second primer set used in this PCR technique (Fve4) the amplifies in intron (3) and exon (4)of F5 gene from NCBI with product size(310bp)which is shown in the figure (3-2).



): PCR product for Fve4 primer, agarose gel electrophoresis (2% agarose gel, ٢Figure (3-minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy) and lineN: from negative control.

) used in this study were previously designed to amplify the F5 gene, ٤Primer (Fve bp).٣١٠show the samples give band in PCR product (

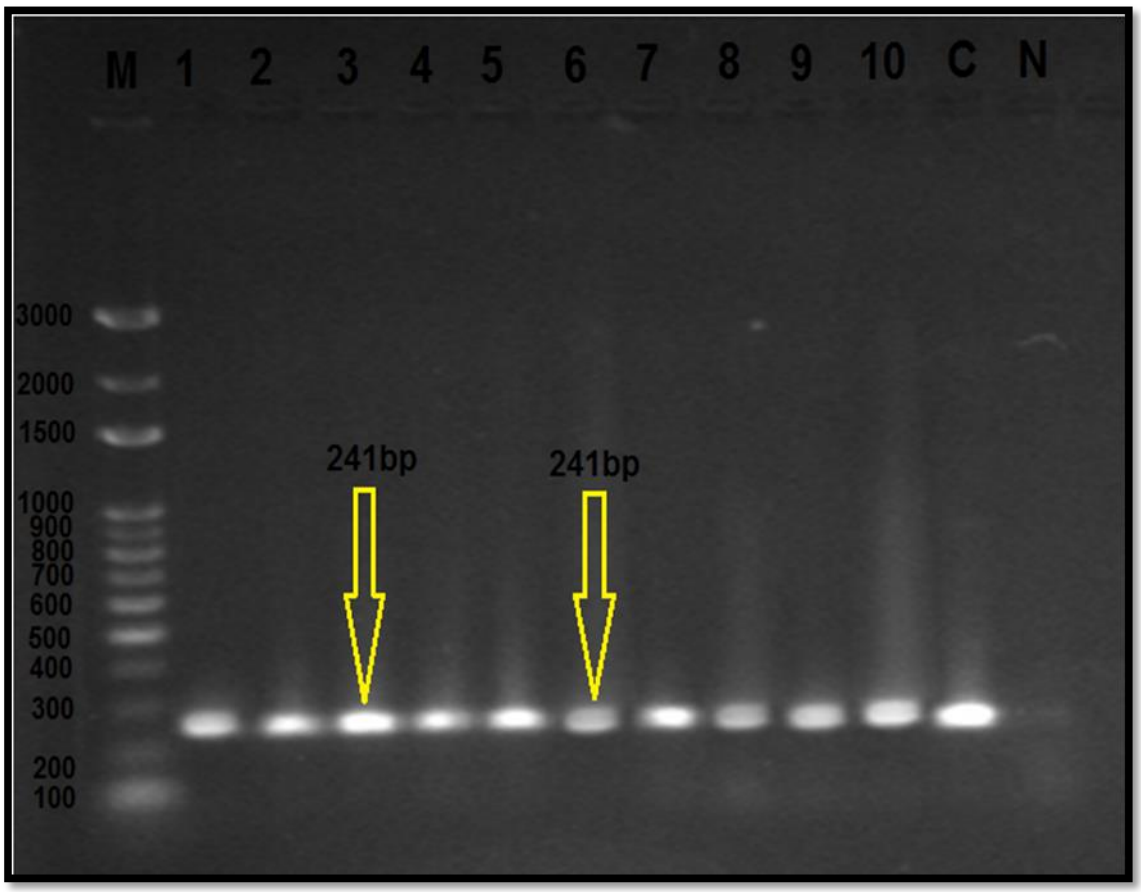
The third primer set used in this PCR technique (Fve6) the amplifies in intron (5),exon (6) and intron (6)of F5 gene from NCBI with product size(547bp)which is shown in the figure (3-3).



): PCR product for Fve6 primer, agarose gel electrophoresis (2% agarose gel, ٣Figure (3-minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy) and lineN: from negative control.

) used in this study were previously designed to amplify the F5 gene, ٦Primer (Fve bp).٥٤٧show the samples give band in PCR product (

The fourthprimer set used in this PCR technique (Fve7) the amplifies in intron (6),exon (7) and intron (7)ofF5 gene from NCBI with product size(241bp)which is shown in the figure (3-4).



): PCR product for Fve7 primer, agarose gel electrophoresis (2% agarose gel, ٤Figure (3-minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy) and lineN: from negative control.

) used in this study were previously designed to amplify the F5 gene, ٧Primer (Fve bp).٤١show the samples give band in PCR product (2

The fifth primer set used in this PCR technique (Fve8) the amplifies in intron (7),exon (8) and intron (8)of F5 gene from NCBI with product size(306bp)which is shown in the figure (3-5).

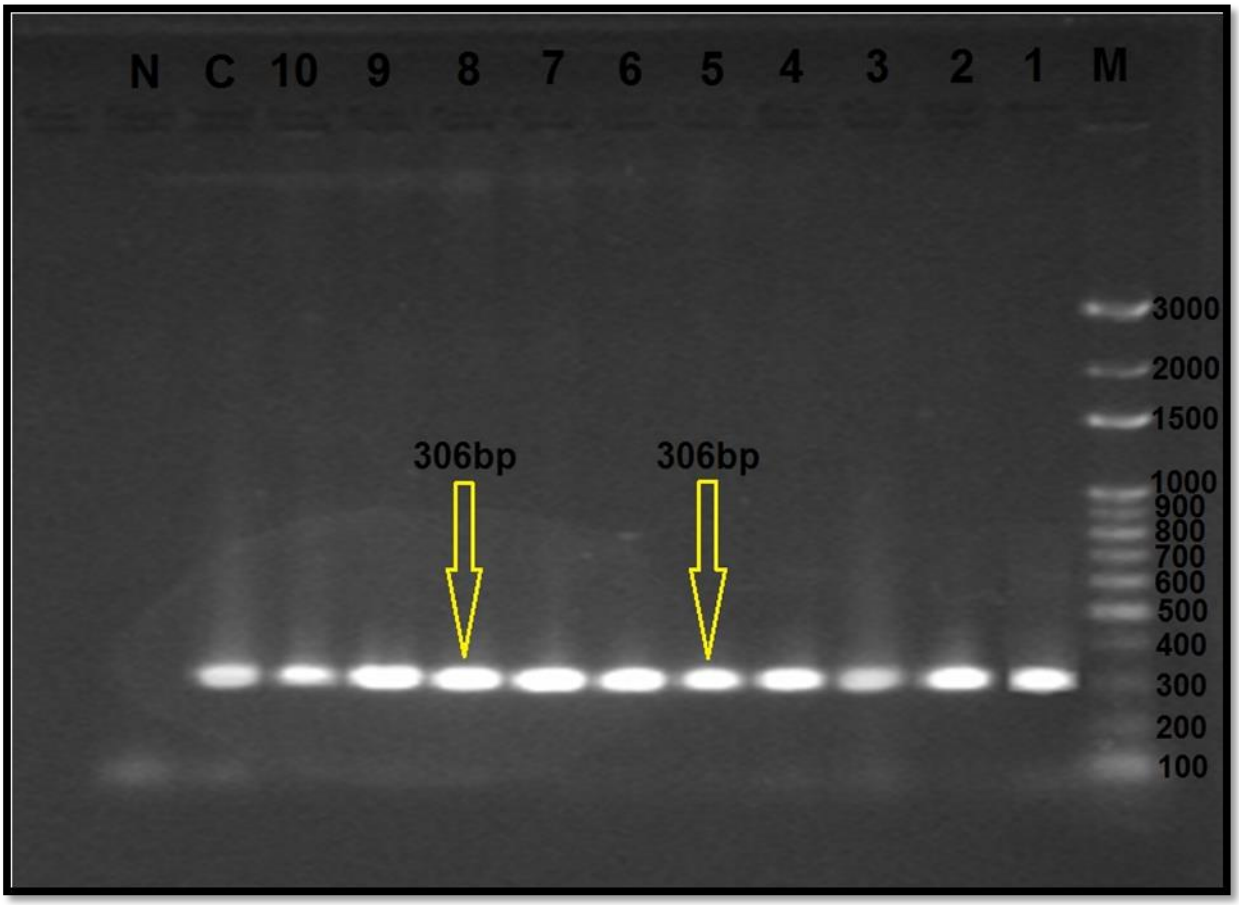
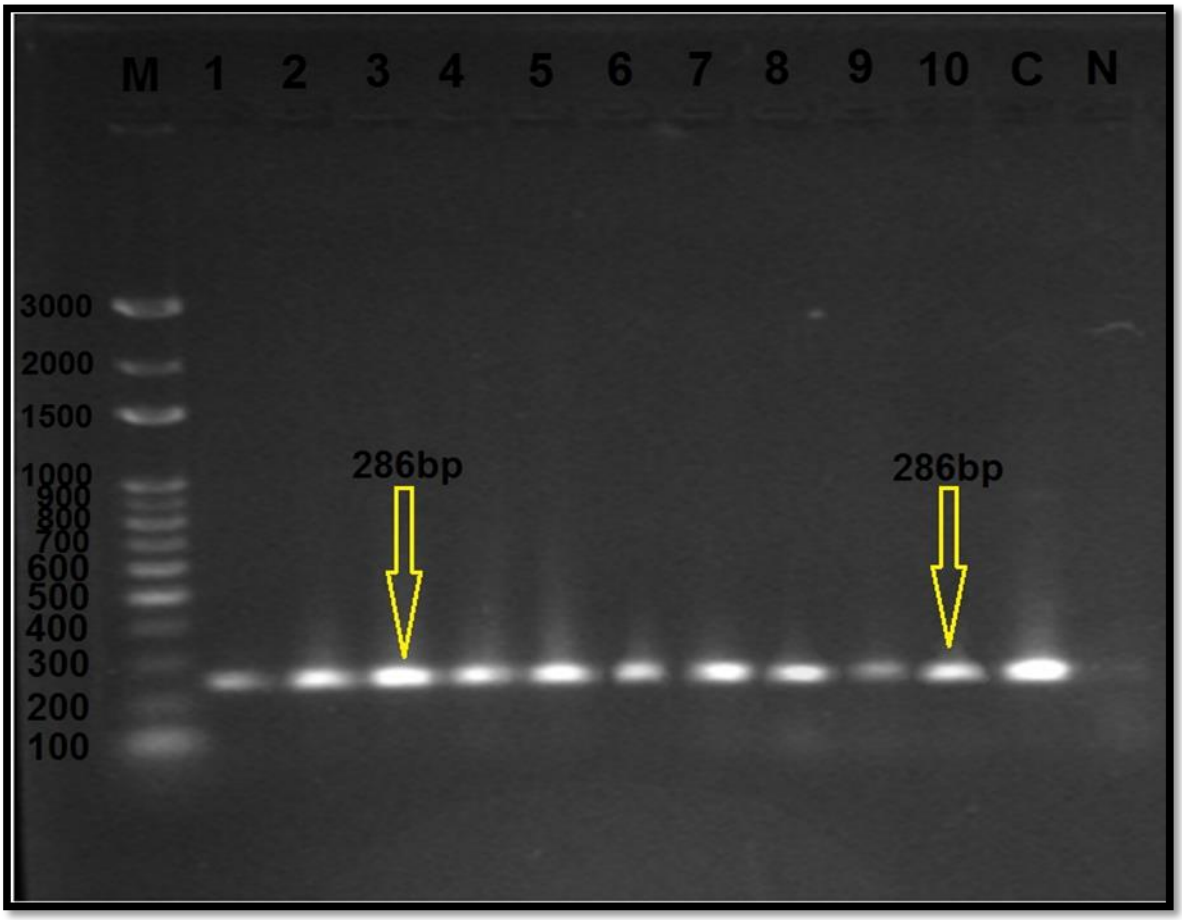


Figure (3-٥): PCR product for Fve8 primer, agarose gel electrophoresis (2% agarose gel, 10 minutes at 100 voltage and then lowered to 70 Volts, ٨٠minutes). Visualized under U.V light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy) andlineN: from negative control.

) used in this study were previously designed to amplify the F5 gene, ٨Primer (Fve bp).٣٠٦show the samples give band in PCR product (

The sixth primer set used in this PCR technique(Fve12)the amplifies in intron (11) and exon (12)of F5 gene from NCBI with product size(286bp)which is shown in the figure (3-6).



): PCR product for Fve12 primer, agarose gel electrophoresis (2% agarose gel, ٦Figure (3-minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy) andlineN: from negative control.

Primer (Fve12) used in this study were previously designed to amplify the F5 gene, show the samples give band in PCR product (286bp).

The seventh primer set used in this PCR technique(Fve13a)the amplifies in intron (12) and exon (13)ofF5gene from NCBI with product length (260bp)which is shown in the figure(3-7).

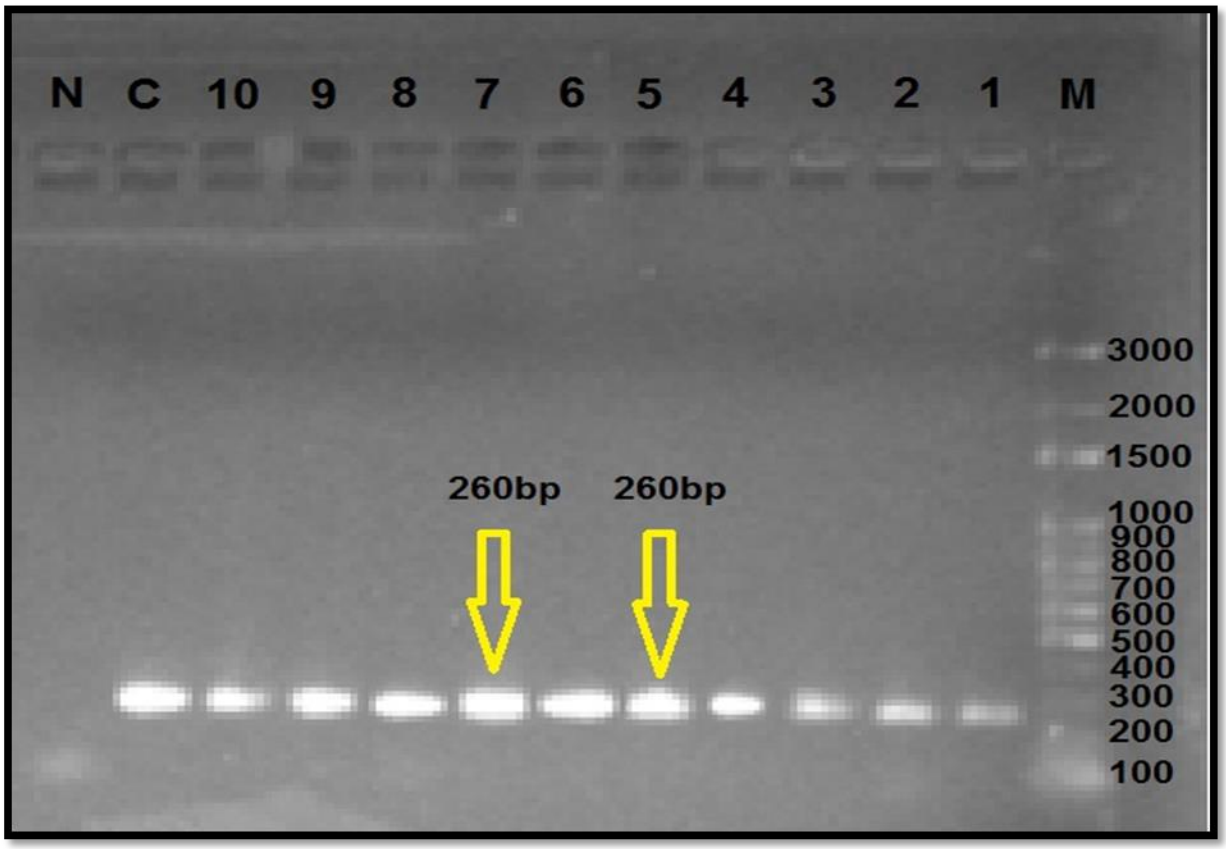


Figure (3-7): PCR product for Fve13a primer, agarose gel electrophoresis (2% agarose gel, minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy) andlineN: from negative control.

Primer (Fve13a) used in this study were previously design to amplify the F5 gene, show the samples give the band in PCR product (260bp).

The eighth primer set used in PCR technique(Fve13c)the amplifies in exon (13) and intron(13)of F5 gene from NCBI with product size (317bp) which is shown in the figure(3-8).

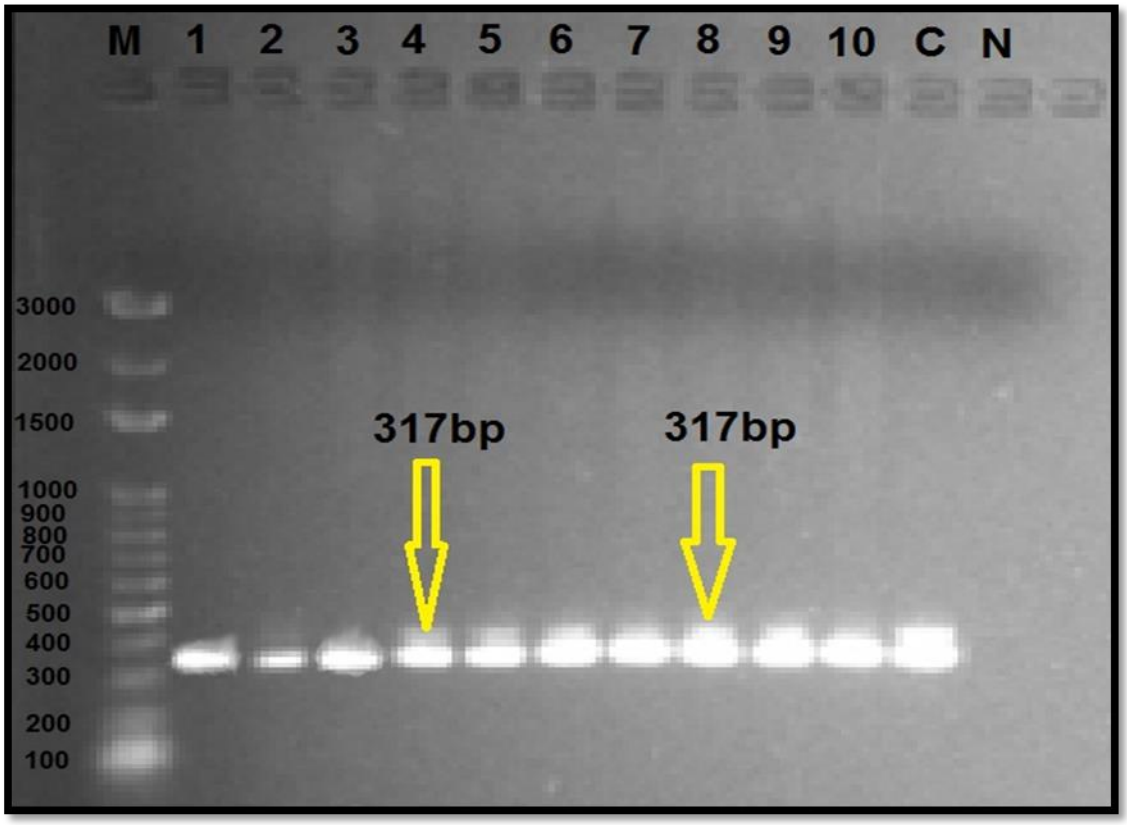
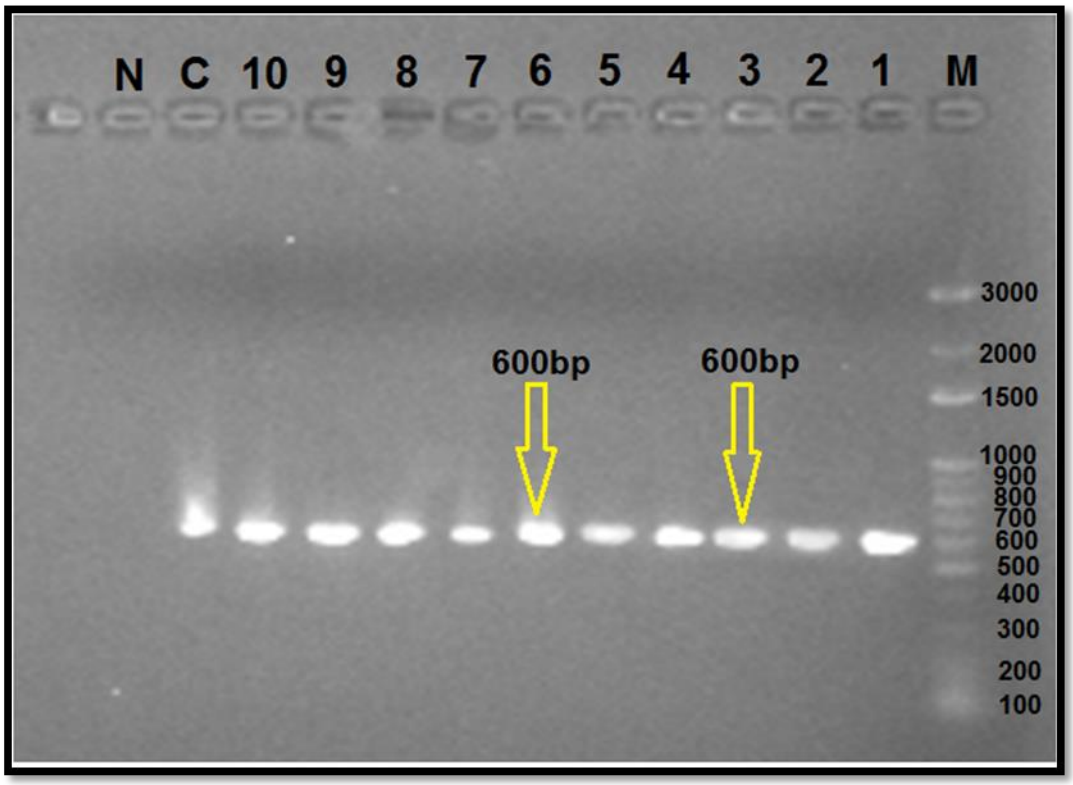


Figure (3-8): PCR product for Fve13c primer, agarose gel electrophoresis (2% agarose gel, minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy)andlineN: from negative control.

Primer Fve13c used in this study were previously design to amplified the F5gene show the samples give the band in PCR product (317bp).

The ninthprimer set used in PCR technique (Fve15)the amplifies in intron(14) ,exon (15) andintron(15)ofF5 gene from NCBI with product size (600bp) which is shown in the figure (3-9).



): PCR product for Fve15 primer, agarose gel electrophoresis (2% agarose gel, ٩Figure (3-minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy)andlineN: from negative control.

Primer Fve1٥used in this study were previously design to amplified the F5gene show the samples give the band in PCR product (٦٠٠bp).

The tenthprimer set used in PCR technique(Fve16)the amplifies in intron (15),exon(16) and intron(16)ofF5gene from NCBI Primer design with product size (333bp)which is shown in the figure(3-10).

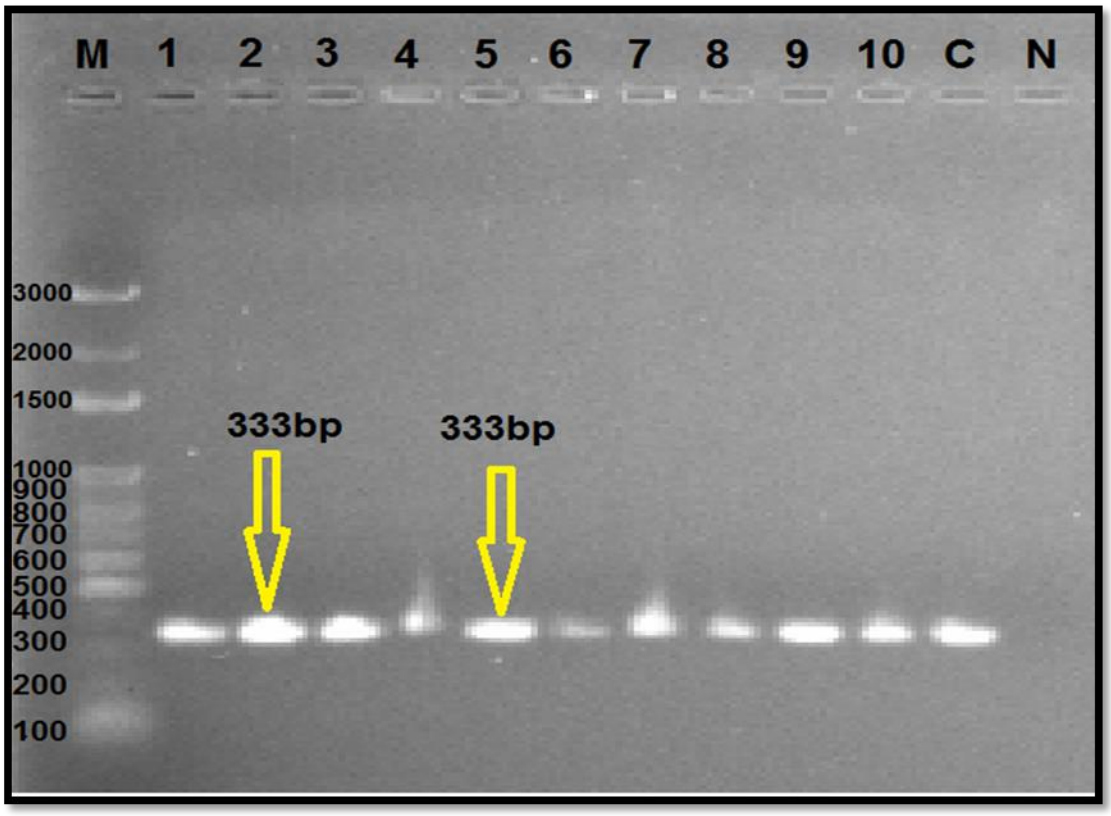


Figure (3-10): PCR product for Fve16 primer, agarose gel electrophoresis (2% agarose gel, minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy)andlineN: from negative control.

Primer Fve16 used in this study were previously design to amplified the F5gene show the samples give the band in PCR product (333bp).

Theeleventh primer set used in PCR technique(Fve25)the amplifies in intron (24)and exon(25) of F5gene from NCBIPrimerdesign with product size(390bp)which is shown in the figure(3-11).

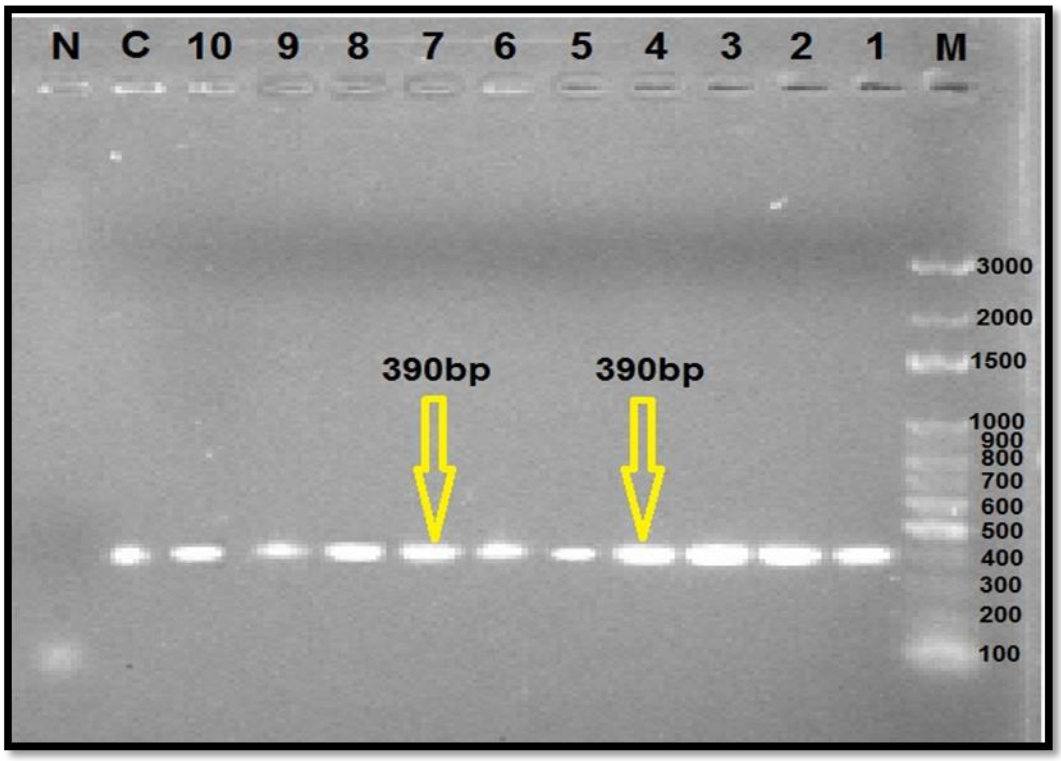


Figure (3-11): PCR product for Fve25 primer, agarose gel electrophoresis (2% agarose gel, minutes). Visualized under U.V ٨٠10 minutes at 100 voltage and then lowered to 70 Volts, light after staining ethidium bromide Line M: 100bp marker,line1-10: from patients,line C: from control (healthy)and lineN: from negative control.

Primer Fve25 used in this study were previously design to amplified the F5gene show all samples give the band in PCR product (390bp).The F5 gene is located on the1q23chromosome. the gene spans 70 kb, consists of 25 exons, and the resulting protein has a relative molecularmass of approximately 330kDa , theheterozygous genotypeswere identified for factor V Leiden mutation in exons of F5 gene (Press*et al*., 2002).

Coagulation factor V is a critical factor of the blood coagulation cascade,this factor circulates in plasma, and isconverted to the active form by the release of the Greer, 2003)according to large (activation peptide by thrombin during coagulation size of the F5gene, many mutations have been identified in the human population, However not all of them are associated with thrombophilia(Yamakage*et al*., 2006). Coagulation factors are a group of related proteins that make up the coagulation system, a series of chemical reactions that form blood clots. After an injury, clots seal off blood vessels to stop bleeding and trigger blood vessel repair,The F5 gene provides instructions for making a protein called coagulation factor V (Asselta*et al*., 2006).

The protein circulates in the bloodstream in an inactive form until the coagulation system is activated by an injury that damages blood vessels.when coagulation factor V is activated, it interacts with coagulation factor X. the active forms of these two coagulation factors (written as factor Va and factor Xa, respectively) form a complex that converts an important coagulation protein called prothrombin to its active form, thrombin. thrombin then converts a protein called fibrinogen into fibrin, which is the material that forms the clot,the factor V protein is made primarily by cells in the liver (Asselta and Peyvandi ,2009).

3.3.Molecular analysis of F5 gene.

Directsequencing of the F5 gene from the all patients in Iraqi populations using primer **Fve12**,**Fve13a**,**Fve13c**,**Fve16** and **Fve25.**

3.3.1. Type of mutations:

The PCR products of thrombosis patients were screened by sequencing. The resultwas directly compared with control (NCBI nucleotide blast) and Mega 6 program.The current utilized forwored primer for sequencing the F5 gene in 30 patients compared with control NCBI nucleotide blast .

In this study, we evaluated the presence mutations in several main exons of F5 gene in individuals which due to thrombosis.

The First DNA sequence of the F5 gene located exon 12 from patient and NCBI nucleotide blast, show the figure (3-12A,B and C).

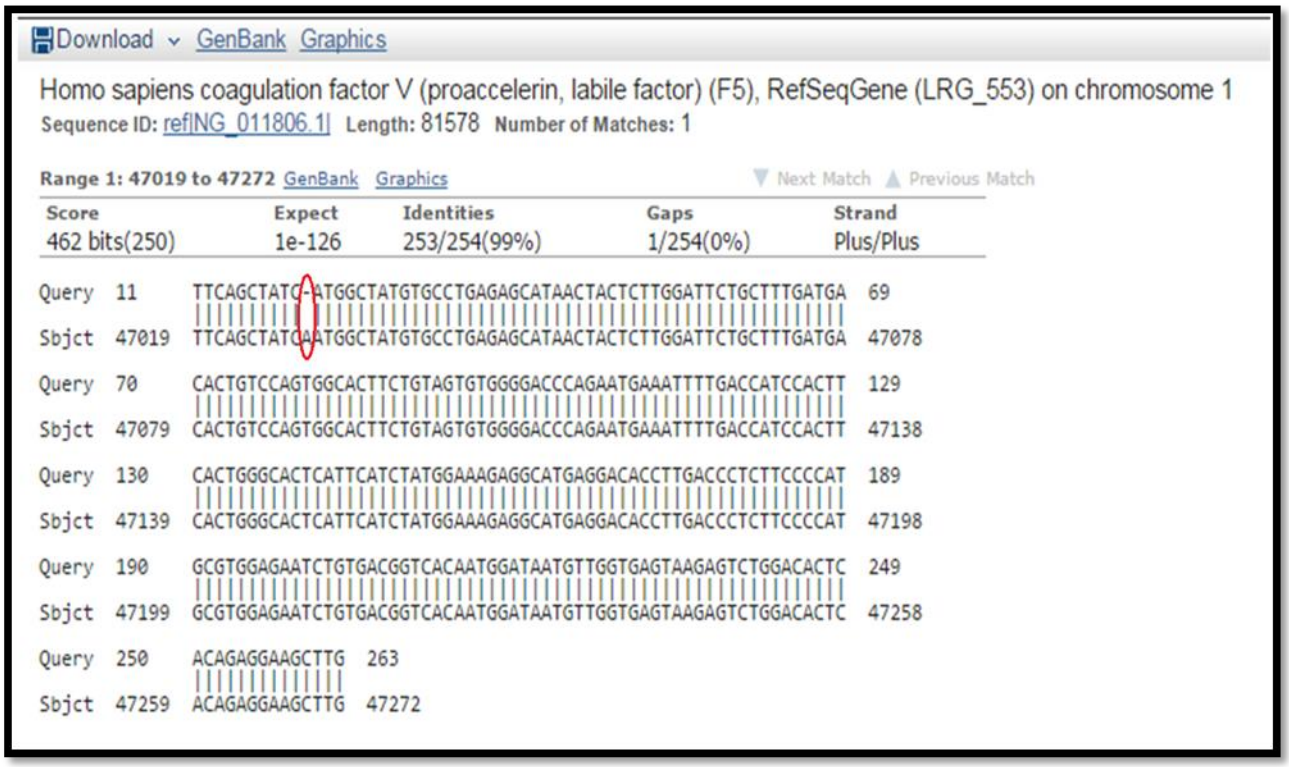


Figure (3-12A): The automated sequencing of F5 genedisplay a deletion in(47029/ A) base pair in Exon(12)of the F5 gene when comparing in the NCBI nucleotide blast.

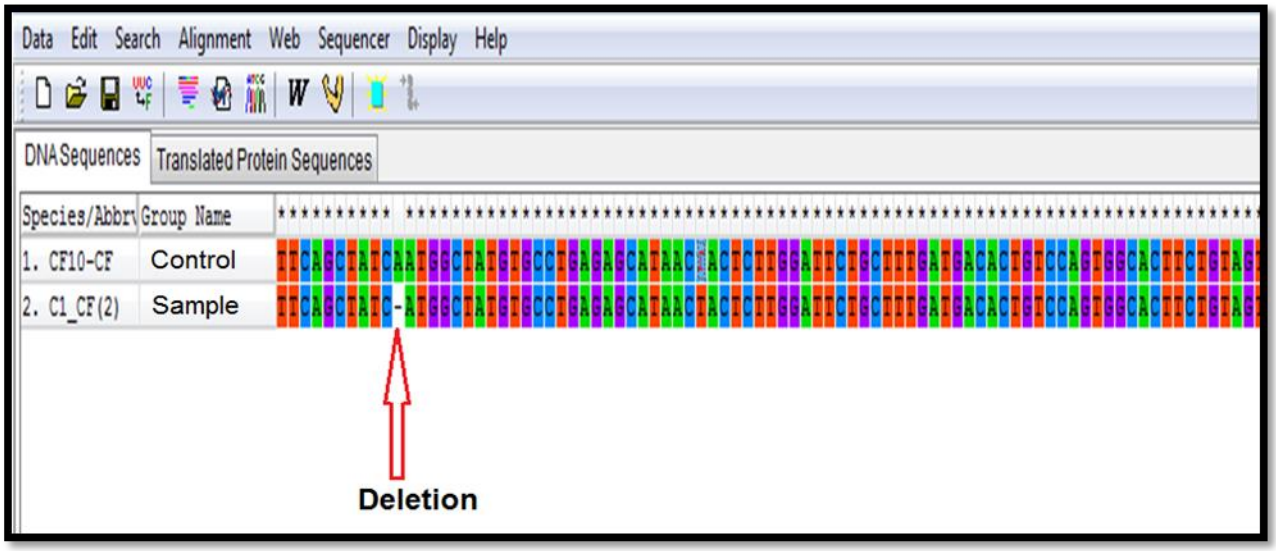
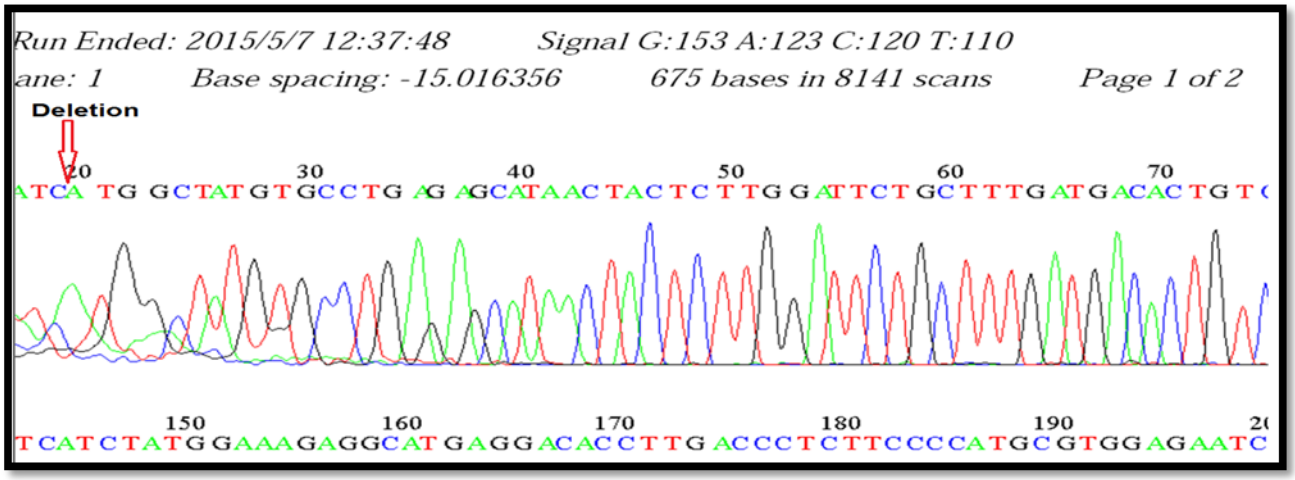


Figure (3-12B) comparing between control (NCBI nucleotideblast) and thrombosis patient in the MEGA 6 program.



TC-ATGGCTATGTGCCTGAGAGCATAACTACTCTTGGATTCTGCTTTGAT GACACTGT

Figure (3-12C): A chromatogram for sample display a sequence and the deletion region.

A sample of primer (Fve12) showed a deletion in nitrogen base 47028-47030/CAA , in exon (12) that caused deletion in amino acid Pro/ del (Zammiti*et al*.,2006).

Deletion of a number of pairs that is not evenly divisible by three will lead to a frameshift mutation, causing all of the codons occurring after the deletion tobe read incorrectly during translation, producing severely altered and potentially nonfunctional protein. In contrast, a deletion that is evenly .divisible by three is called an in-frame deletion (Ren, 2005)

The Second DNA sequence of the F5 gene located exon 13 from patient and NCBI nucleotide blast, show the figure (3-13A,B and C).

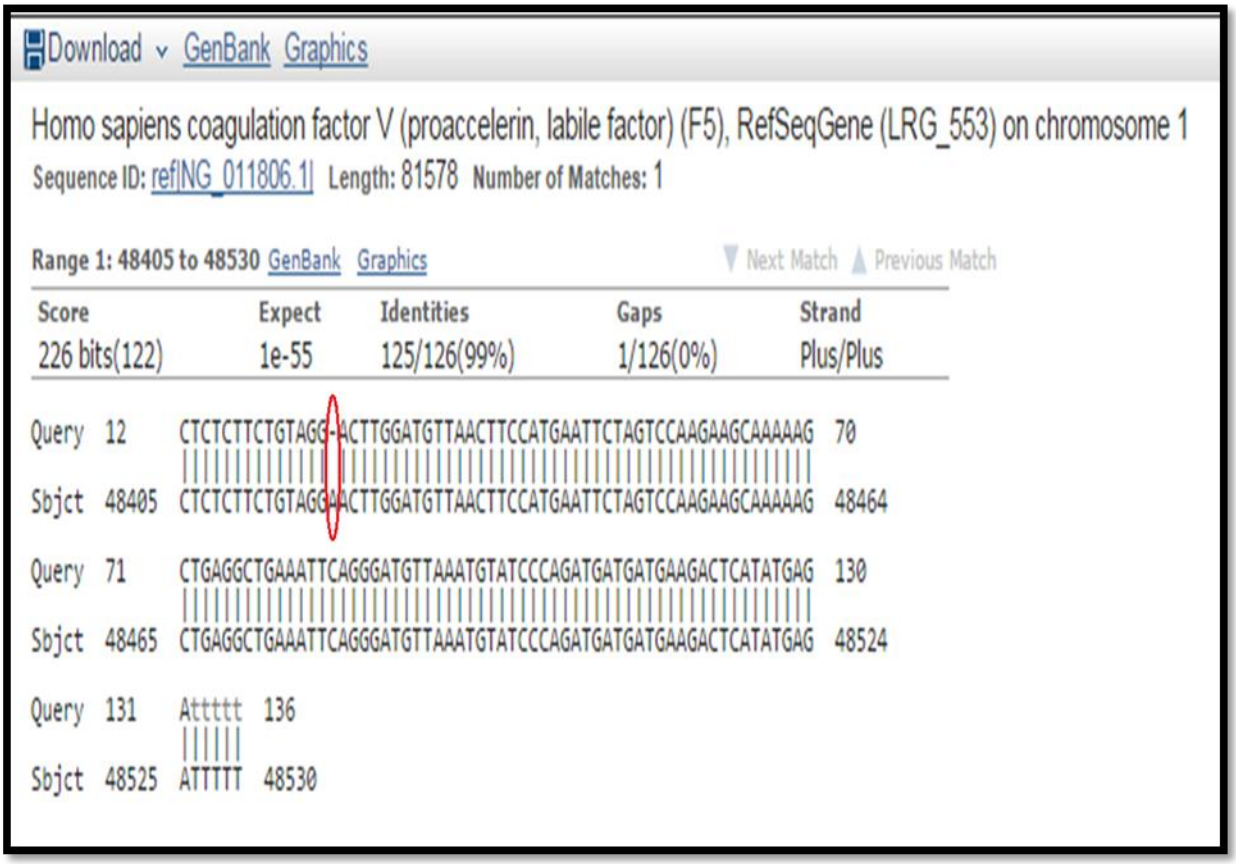
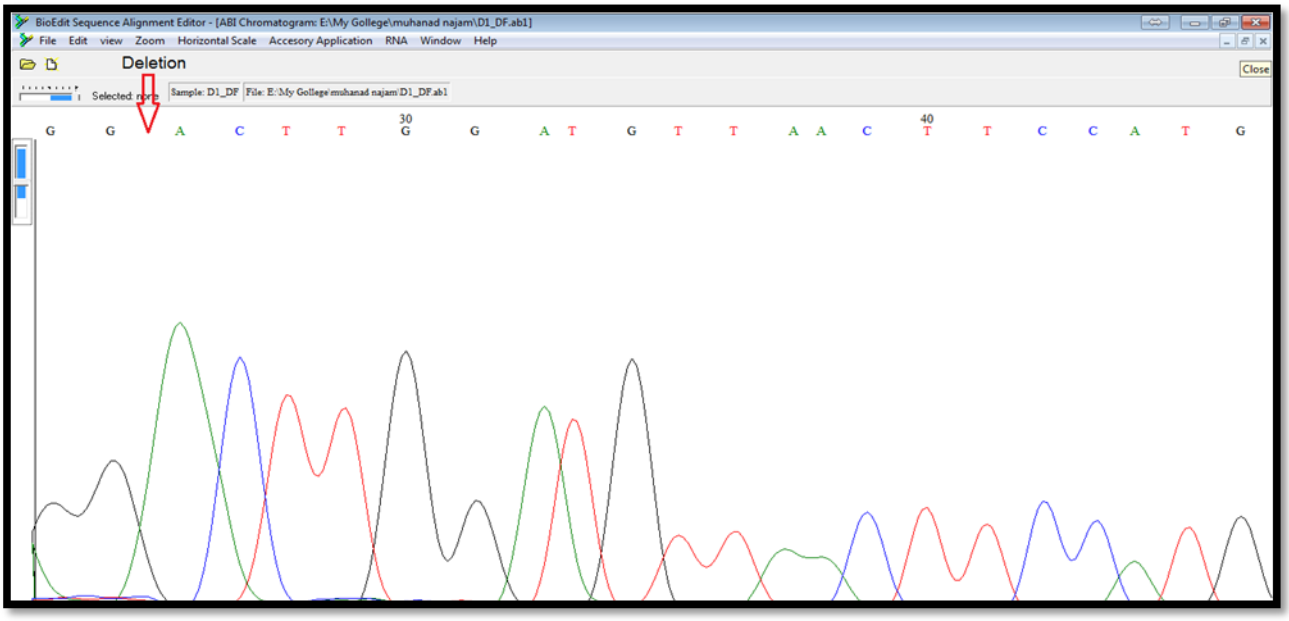


Figure (3-13A):The automated sequencing of F5 genedisplay a deletion in (48419/ A) base pair in Exon (13) of the F5 gene when comparing in the NCBI nucleotide blast.

Figure (3-13B) comparing between control (NCBI nucleotideblast) and thrombosis patient in the MEGA 6 program.



GG-ACTTGGATGTTAACTTCCATG

Figure (3-13C): A chromatogram for sample thrombosis patient display a sequence and the deletion region.

Asampleofprime(Fve13a)showedadeletioninnitrogenbase48418-48420/CAA, in exon (13) that caused deletion in amino acid Ser/del.DNA sequence analysis revealed causative mutations in heterozygous form base deletion in exon (13) (Montefusco *et al*.,2000).

A mutation in which a part of a chromosome or a sequence of DNA is lost during DNA replication. Any number of nucleotides can be deleted, from a single base to an entire piece of chromosome(Lewis, 2004).

The Thrird DNA sequence of the F5 gene located exon 13 from patient and NCBI nucleotide blast, show the figure (3-14A,B and C).

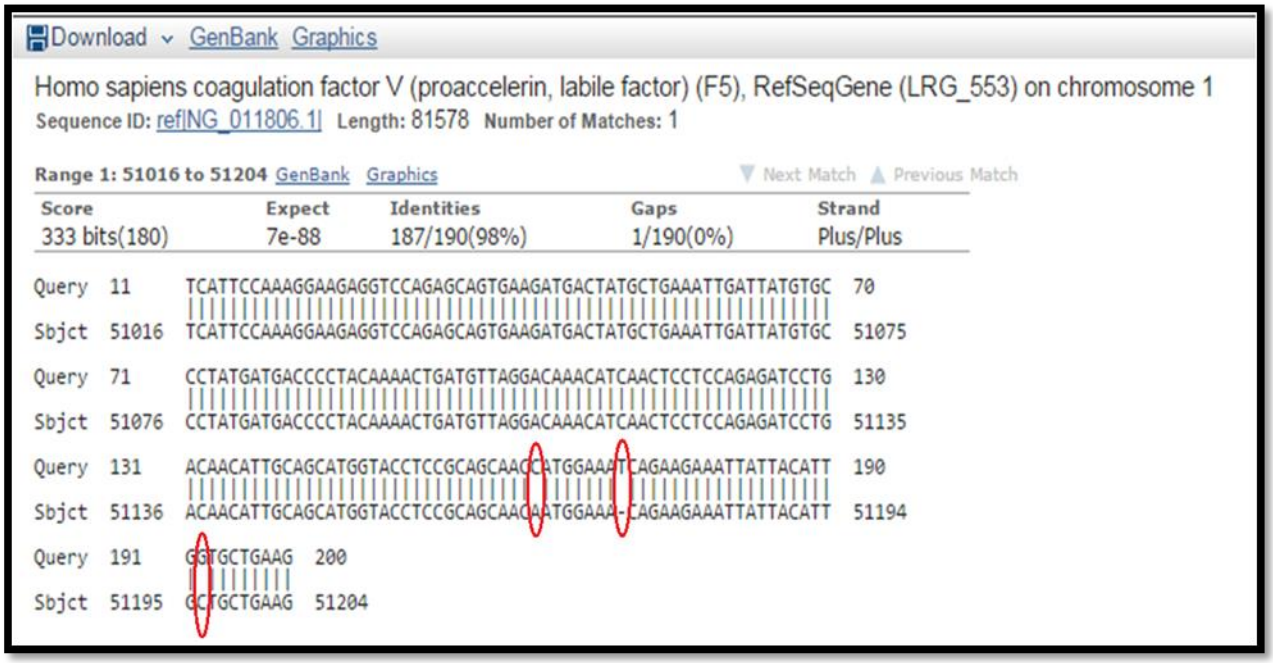


Figure (3-14A):The automated sequencing of F5 genedisplay a substitution in (51168/C/A),isertion in (51176/T) and substitution in(51196/G/C) base pair in Exon (13) of the F5 gene when comparing in the NCBI nucleotide blast.

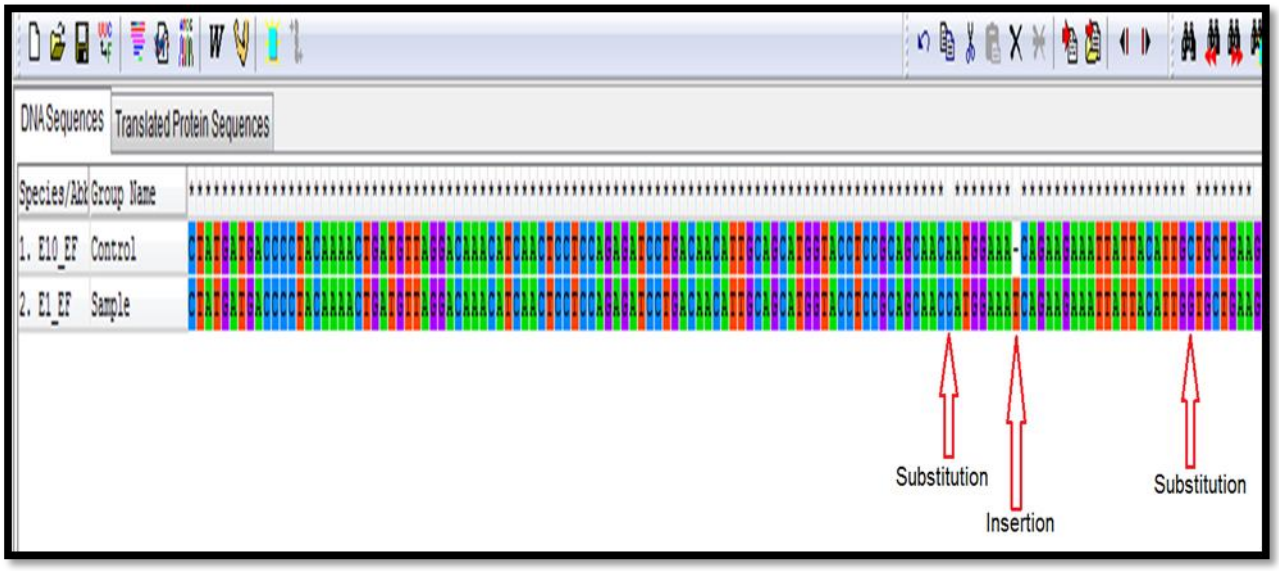
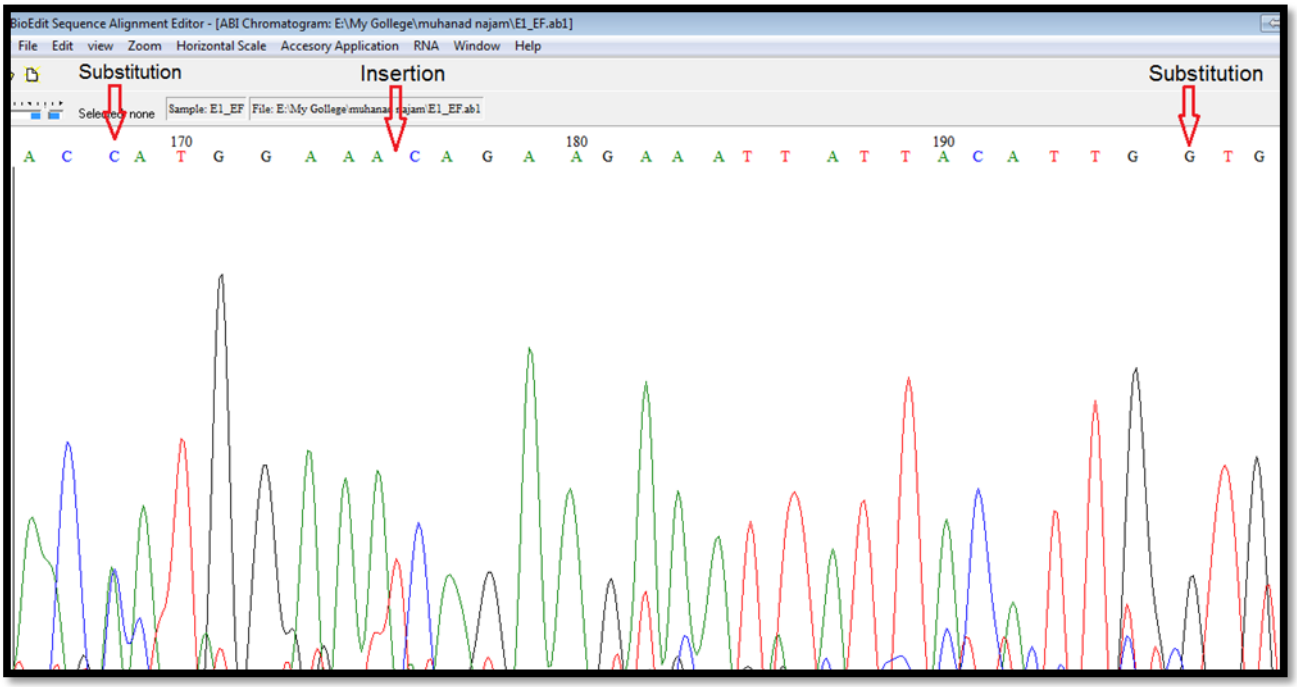


Figure (3-14B) :comparing between control (NCBI nucleotideblast) and thrombosis patient in the MEGA 6 program.



ACCATGGAAATCAGAAGAAATTATTACATTGGTG

Figure (3-14C): A chromatogram for sample thrombosis patient display a sequence and the substitution and insertion regions.

In thesample(I) of primer (Fve13c) was showa SNP assubstitution A / C innitrogenbase51148-51150/CAA, in exon (13) that caused a Missense mutation because its coded for amino acid Gln/Pro(astoldi*et al*.,2000).

A sample (II)ofprime(Fve13c) showed insertion (T) innitrogenbase 51157that caused insertion in amino acidHis /Ser(Kling*etal*.,2006),and sample (III) ofprime(Fve13c) was show a SNP as substitution C / Ginnitrogenbase 51195-51197/GCT, in exon (13) that caused a Missense mutation because its coded for amino acid Ala/Gly (Huang*et al*., 2010).

The Fourth DNA sequence of the F5 gene inlocated exon16 and intron16 from patient and NCBI nucleotide blast, show the figure (3-15A,B and C).

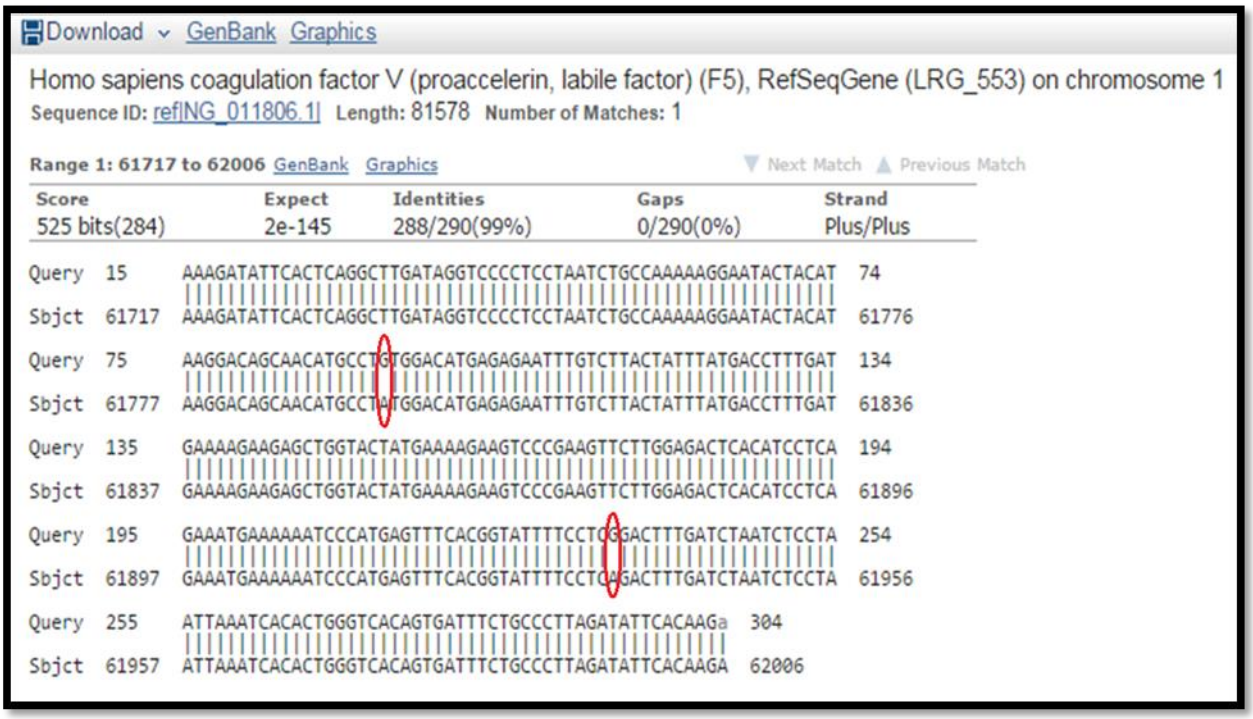
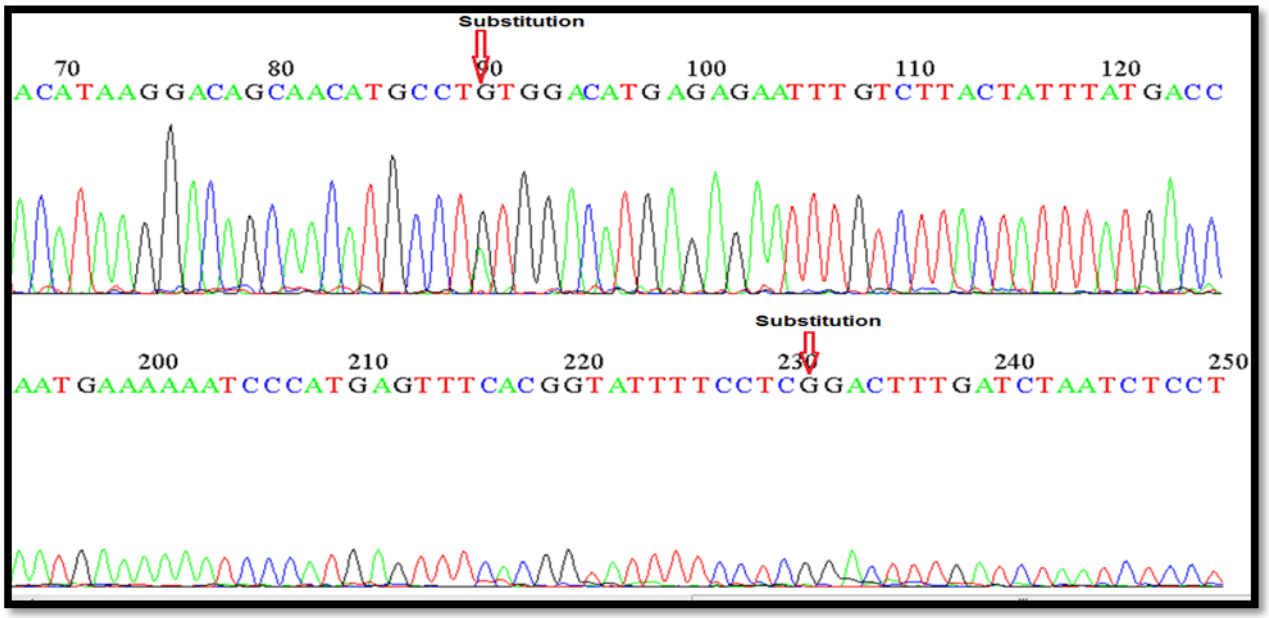


Figure (3-15A):The automated sequencing of F5 genedisplay a substitution in (61795/G/A) base pair in Exon (16) and substitution in (61936/G/A) base pair in Intron (16) of the F5 gene when comparing in the NCBI nucleotide blast.

Figure (3-15B) : comparing between control (NCBI nucleotideblast) and thrombosis patient in the MEGA 6 program.



ACATAAGGACAGCAACATGCCTGTGGACATGAGAGAATTTGTCTTAC

TATTTATGACC

AATGAAAAAATCCCATGAGTTTCACGGTATTTTCCTCGGACTTTGATC
TAATCTCCT

Figure (3-15C): A chromatogram for sample thrombosis patient display a sequence and the substitution regions.

In thesample(I) of primer (Fve16)was show a SNP as substitution A / G innitrogenbase61794-61796/TAT, in exon (16) that caused a Missense mutation because its coded for amino acid Tyr / Cys(Lunghi*et al* .,2005).

Factor V Leiden mutation is a point mutation in factor V gene which is obtained by the substitution of adeninefor guanine at nucleotide position 1691,were homozygous for 48571 A>G missense mutation in 16 exon(Ali Nazemi*et al*.,2013)

.

Interest in the genetic basis of thrombosis was accelerated with the discoveryof the factor V Leiden (FVL) mutation,which is considered the most common genetic risk factor(Rosendaal , 1999).

The Fifth DNA sequence of the F5 gene inlocated exon25 from patient and NCBI nucleotide blast, show the figure (3-16A,B and C).

Figure (3-16A):The automated sequencing of F5 genedisplay insertion in (77362/A) base pair in exon 25 of the F5 gene when comparing in the NCBI nucleotide blast.

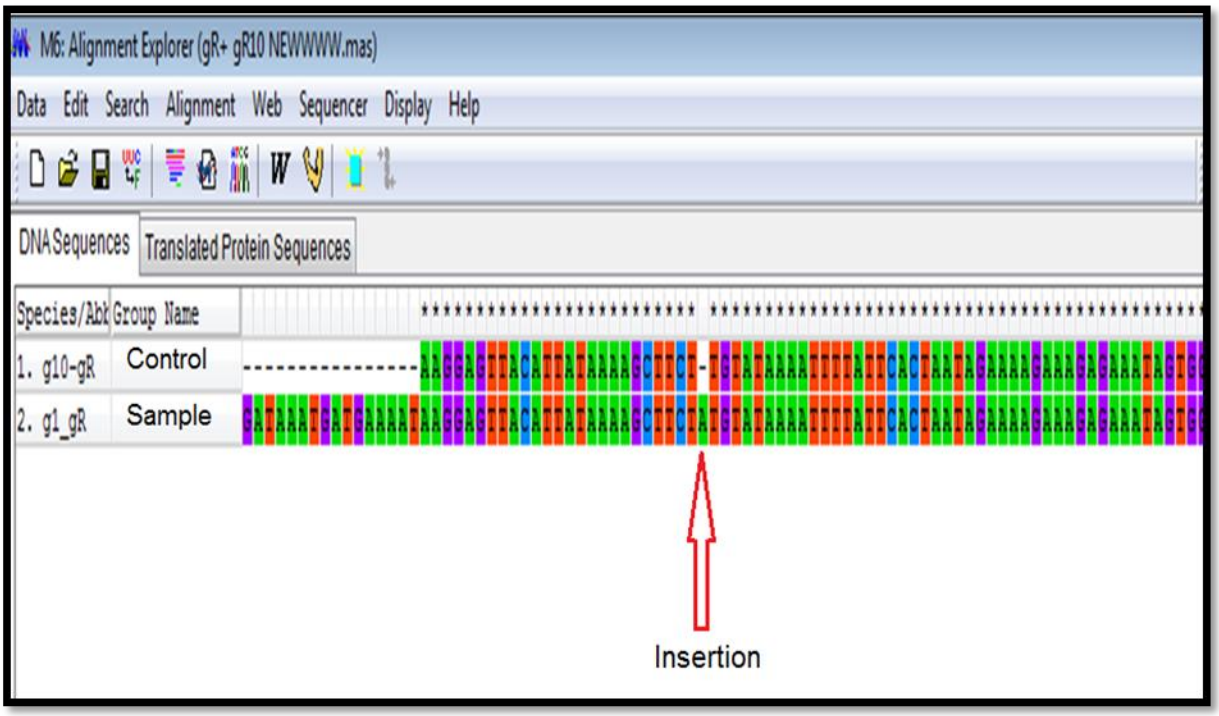
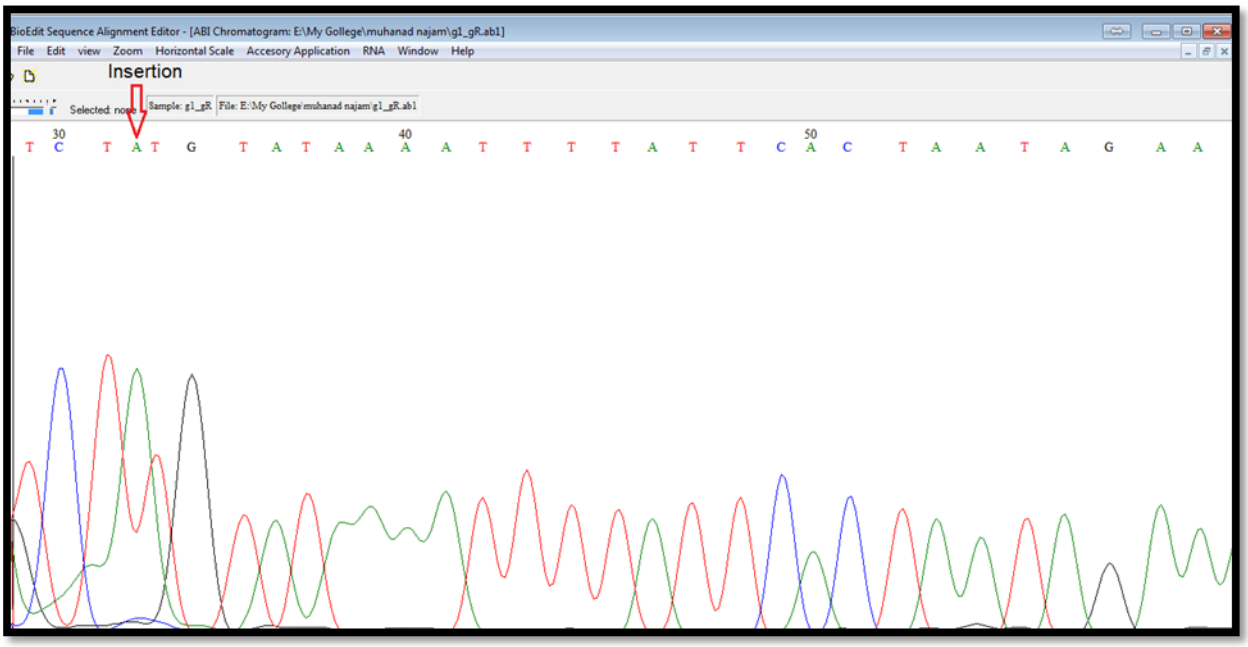


Figure (3-16B): comparing between control (NCBI nucleotideblast) and thrombosis patient in the MEGA 6 program.



TCTATGTATAAAATTTTATTCACTAATAGAA

Figure (3-16C): A chromatogram for sample thrombosis patient display a sequence and the insertion region.

A sample (I)ofprime(Fve 25) showed insertion (A) innitrogenbase 77353that caused insertion in amino acidCys/Met(Shinozawa*et al*.,2007).

Different mutation of one or more than located gene region. However point mutation , substitution , insertion and deletion affected the F5 gene in Iraqi patients.as show in table (3-6).

Table (3-6):point mutations detect in patient samples.

Name of Primers	Wild type	Mutant type	Change in amino acids	Site Of N.A.	Type of Mutation	Effect on translation
(Fve12)	CAA	-AT	Pro_Deletion	21	Deletion	Frame shift
(Fve13a)	GAA	-AC	Ser_Deletion	26	Deletion	Frame shift
(Fve13C)	CAA	CCA	Gln- Pro	163	Substitution	Missense
	C AC	TCA	His- Ser	171	Insertion	Frame shift
	GCT	GGT	Ala- Gly	192	Substitution	Missense
(Fve16)	TAT	TGT	Tyr- Cys	89	Substitution	Missense
(Fve25)	TGT	ATG	Cys- Met	44	Insertion	Frame shift

Different genetic variants within a species are referred to as alleles, and so a new mutation is said to create a new allele. each allele is characterized by a selection coefficient, which measures the expected change in an allele's frequency over time (Wielgoss*et al*., 2011).

The direct sequencing of the of F5 gene located in exon 12, exon 13 ,exon 16, intron 16 and exon 25 change the position in 21,26,163, 171, 192, 89and 44 these different type mutation caused the factor V gene.this result agree with those of (Montefusco *et al*.,2000 ;Shinozawa*et al*.,2007 and Ali Nazemi*et al*.,2013).

3.3.2. Percentage of mutations.

The rate at which various types of mutations occur over time. mutation rates are typically given for a specific class of mutation, for instance point mutations, small or large scale insertions or deletions. The rate of substitutions can be further subdivided into a mutation spectrum which describes the influence of genetic context on the mutation rate(Ossowski*et al*. 2010).

Analysis of F5 gene by sequencing for Iraqi patients exhibited the existence of many genetic variation. three types of mutations namely deletion , insertionandsubstitutionwere present. percentage of mutationtypes that showed 25% for deletion , 37.5% for substitution and 25% for insertion.as show in table (3-7).

Table (3-7): percentage of mutation type.

Type of Mutation	***Percentage%***
Substitution	42.86%

Insertion	28.57%
Deletion	28.57%

3.3.3.Effect of mutations.

Mutation can result in several different types of change in sequences of F5 gene.point mutations typically refer to alterations of single base pairs of DNA or to a small number of adjacent base pairs. in this section, we shall consider the effect of such changes at the phenotypic level. Point mutations are classified in molecular, which shows the main types of DNA changes and their functional effects at the protein level(Freeman and Company,2000).

Table (3-8) shows that the substitution mutation was a missense (42.86%) causing impact on phenotype that leads to replacement in amino acid ,the deletion and insertion mutation lead to frame shift which represented 57.14% usually introduces premature stop codons in addition to lots of amino acid changes. in this study. These mutations result in a completely different translation in F5gene.

Table (3-8) : percentage of effect of mutation .

mutation	***Percenage%***
Frame shift	**57.14%**
Missense	**42.86%**

If a mutation changes a protein produced by a gene, the result is likely to be harmful, with an estimated 70 percent of amino acid polymorphisms that have damaging effects, and the remainder being either neutral or weakly beneficial(Sawyer *et al*.,2007).

Chapter Four

Conclusions

and

Recommendation

٤ .Conclusions and recommendations.

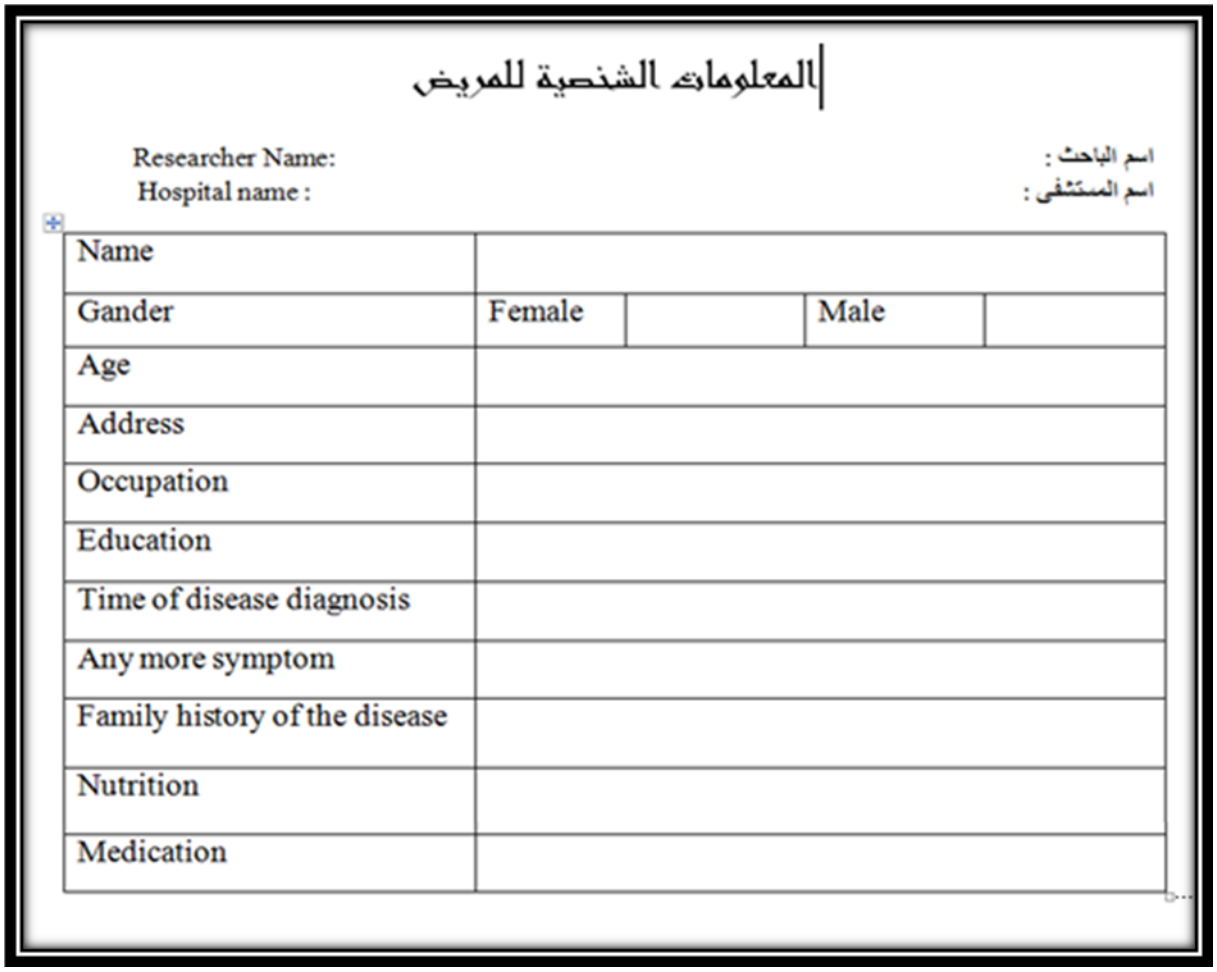
4.1. Conclusion.

1. The age group between 50 to 60 are more susceptible to thrombosis .
2. The thrombosis was more frequent in male than female.
3. Change in DNA was mostly SNP. This change was in three type (Substitution ,Insertion , Deletion).
4. Leiden mutation was confirmed in this group of Iraqi patients.
5. Pre diagnosis in members of families with heart strock history is necessary and should be supported by molecular detection .

4.2. Recommendation.

1. Detection of other types of mutation by amplifying another regions of the gene by using other primers.
2. Investigating the role of G-protein receptor as an effects in inducing thrombosis.
3. Purification of G-protein from all groups of patients and control by ion exchange chromatography and gel filtration chromatography.

Appendix (I):Case profile



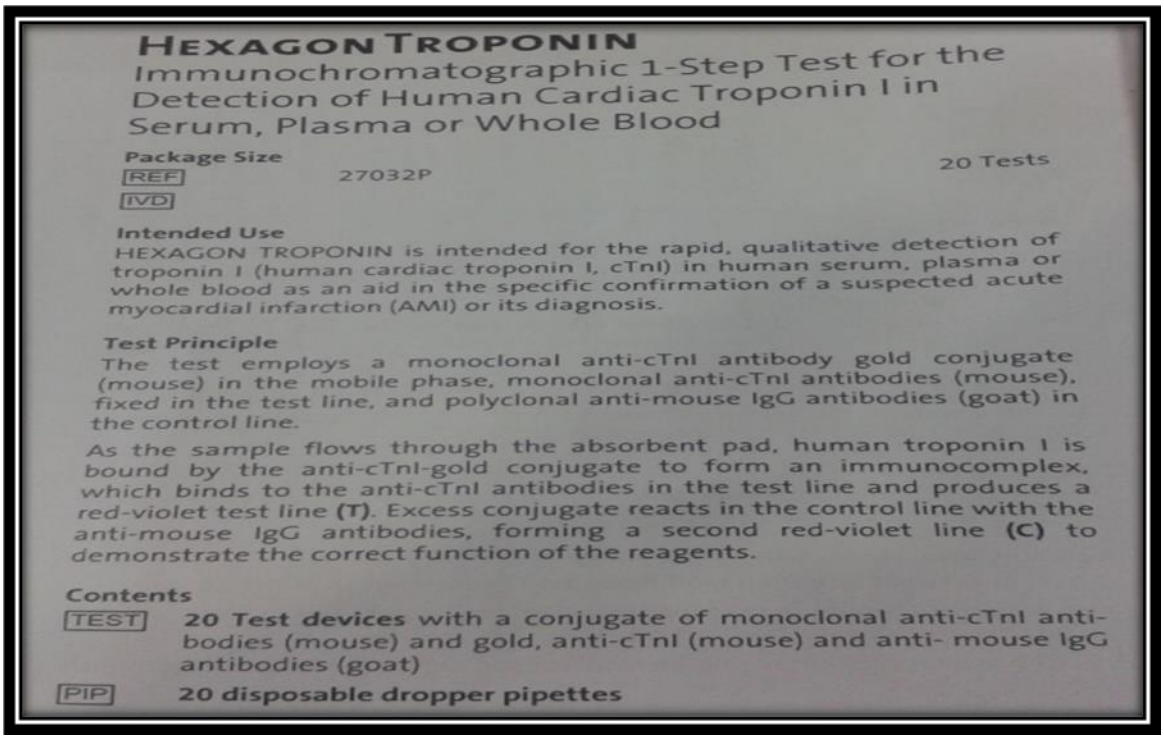
المعلومات الشخصية للمريض

Researcher Name: اسم الباحث :

Hospital name : اسم المستشفى :

Name				
Gander	Female		Male	
Age				
Address				
Occupation				
Education				
Time of disease diagnosis				
Any more symptom				
Family history of the disease				
Nutrition				
Medication				

Appendix (II): Procedure of test troponin.



HEXAGON TROPONIN

Immunochromatographic 1-Step Test for the Detection of Human Cardiac Troponin I in Serum, Plasma or Whole Blood

Package Size 20 Tests

REF 27032P

IVD

Intended Use

HEXAGON TROPONIN is intended for the rapid, qualitative detection of troponin I (human cardiac troponin I, cTnI) in human serum, plasma or whole blood as an aid in the specific confirmation of a suspected acute myocardial infarction (AMI) or its diagnosis.

Test Principle

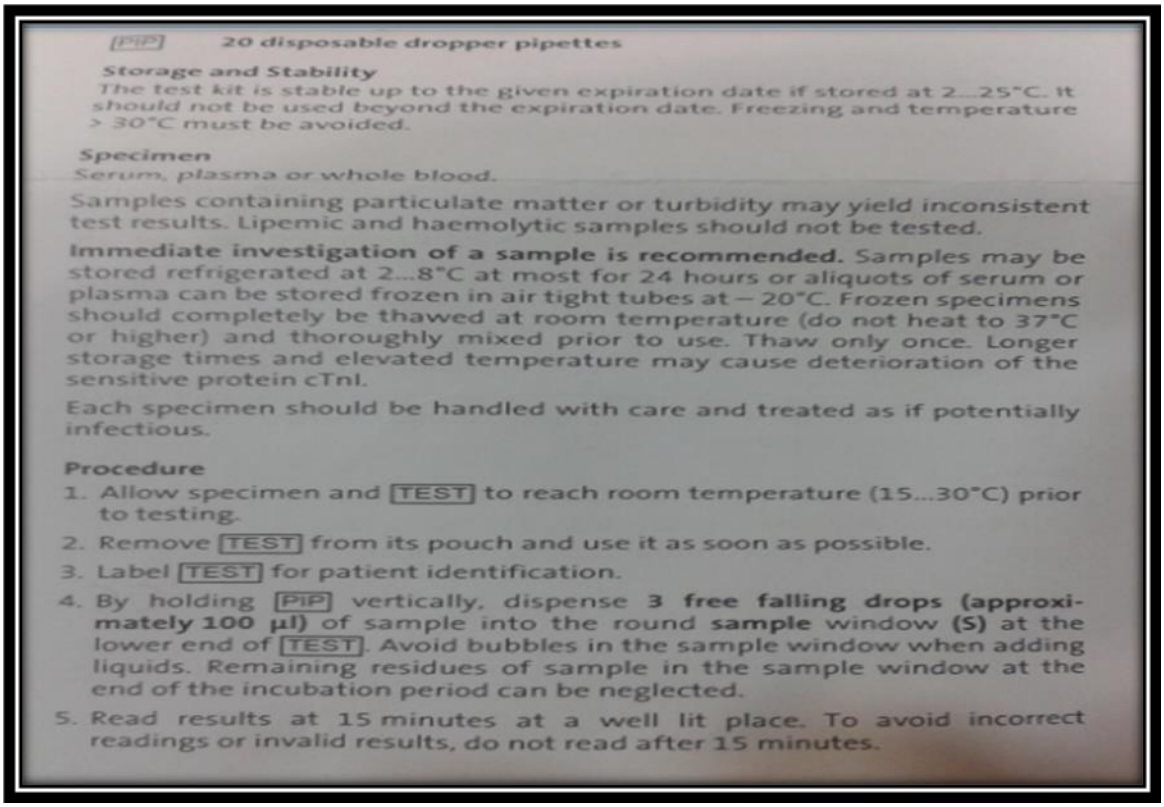
The test employs a monoclonal anti-cTnI antibody gold conjugate (mouse) in the mobile phase, monoclonal anti-cTnI antibodies (mouse), fixed in the test line, and polyclonal anti-mouse IgG antibodies (goat) in the control line.

As the sample flows through the absorbent pad, human troponin I is bound by the anti-cTnI-gold conjugate to form an immunocomplex, which binds to the anti-cTnI antibodies in the test line and produces a red-violet test line **(T)**. Excess conjugate reacts in the control line with the anti-mouse IgG antibodies, forming a second red-violet line **(C)** to demonstrate the correct function of the reagents.

Contents

TEST **20 Test devices** with a conjugate of monoclonal anti-cTnI antibodies (mouse) and gold, anti-cTnI (mouse) and anti- mouse IgG antibodies (goat)

PIP **20 disposable dropper pipettes**



PIP **20 disposable dropper pipettes**

Storage and Stability

The test kit is stable up to the given expiration date if stored at 2...25°C. It should not be used beyond the expiration date. Freezing and temperature > 30°C must be avoided.

Specimen

Serum, plasma or whole blood.

Samples containing particulate matter or turbidity may yield inconsistent test results. Lipemic and haemolytic samples should not be tested.

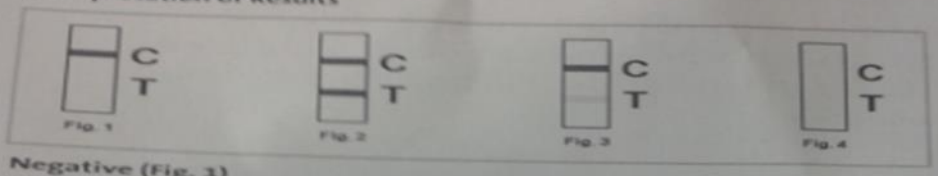
Immediate investigation of a sample is recommended. Samples may be stored refrigerated at 2...8°C at most for 24 hours or aliquots of serum or plasma can be stored frozen in air tight tubes at – 20°C. Frozen specimens should completely be thawed at room temperature (do not heat to 37°C or higher) and thoroughly mixed prior to use. Thaw only once. Longer storage times and elevated temperature may cause deterioration of the sensitive protein cTnI.

Each specimen should be handled with care and treated as if potentially infectious.

Procedure

1. Allow specimen and TEST to reach room temperature (15...30°C) prior to testing.
2. Remove TEST from its pouch and use it as soon as possible.
3. Label TEST for patient identification.
4. By holding PIP vertically, dispense **3 free falling drops (approximately 100 µl)** of sample into the round **sample** window **(S)** at the lower end of TEST. Avoid bubbles in the sample window when adding liquids. Remaining residues of sample in the sample window at the end of the incubation period can be neglected.
5. Read results at 15 minutes at a well lit place. To avoid incorrect readings or invalid results, do not read after 15 minutes.

Interpretation of Results



Negative (Fig. 1)
Only one red-violet Control line **(C)** appears in the upper part of the rectangular result window showing that the test has been carried out correctly.

Positive (Fig. 2 and 3)
A second red-violet Test line **(T)** appearing in the lower part of the rectangular result window, indicates a positive result for cardiac Troponin I in the sample.

Even a weak line indicates a positive result (Fig. 3).

Different intensities between Test **(T)** and Control **(C)** lines may occur but are irrelevant for the interpretation of the results.

***Invalid* (Fig. 4)**
If no control line appears, even if a test line is visible, the test has to be repeated with a fresh TEST.

Performance Characteristics
The test detects troponin I with a sensitivity limit of about 0.5 ng/ml.

No cross-reactivity has been observed with other troponins (troponin T, skeletal troponin I).

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/1c-tropi.pdf or

www.human-de.com/data/gb/vr/1c-tropi.pdf

Performance Characteristics
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www.human.de/data/gb/vr/1c-tropi.pdf or

www.human-de.com/data/gb/vr/1c-tropi.pdf

Notes

1. cTnI is highly specific for cardiac injury.
2. After cardiac injury, troponin I is released into the blood 4-8 hours after the onset of pain and remains elevated for 6-10 days.
3. The test cannot detect less than 0.5 ng/ml troponin I in a specimen. A negative result does not preclude the possibility of myocardial infarction. If AMI is suspected the test should be repeated at appropriate intervals within the first 12 hours after onset of symptoms. Test results must always be evaluated with other data and information available to the physician.
4. All materials contaminated with patient specimens should be inactivated by validated procedures (autoclaving or chemical treatment) according to applicable regulations.

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الإهداء

يامن أحسن كل شيء خلقه...يامن أبدع صنع كل شيء... يامن بيده ملكوت السموات والارض **إليك ربي**

إلى رمز العزة.. والشموخ. ومنقذ البشرية.. من الجهل وباني مدينه العلم**الرسول الأعظم محمد (ﷺ)**

إلى أبي.....

وإلى أمي...

أقول لهم: أنتم وهبتموني الأمل والنشأة على شغف الاطلاع والمعرفة

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

إِنَّا فَتَحْنَا لَكَ فَتْحًا مُبِينًا ﴿١﴾ لِيَغْفِرَ لَكَ اللَّهُ مَا تَقَدَّمَ مِنْ ذَنْبِكَ وَمَا تَأَخَّرَ وَيُتِمَّ نِعْمَتَهُ عَلَيْكَ وَيَهْدِيَكَ صِرَاطًا مُسْتَقِيمًا ﴿٢﴾ وَيَنْصُرَكَ اللَّهُ نَصْرًا عَزِيزًا ﴿٣﴾

صدق اللَّهُ العظيم

﴿ سورة الفتح ﴾

الخلاصة

تهدف هذه الدراسة الى التحري عن دور المورث F5 ذو الطفرة الوحيدة في التسبب في الجلطة القلبية وتشخيص طفرة لايدن عند المرضى العراقيين المصابين بالجلطة الدموية .

عينات الدم جمعت من ٤٠ مريض من الفترة تشرين الثاني ٢٠١٤ – كانون الأول ٢٠١٥ متواجدون في غرفة العناية المركزة في مستشفيات اليرموك التعليمي والكاظمية التعليمي إضافة إلى ١٠ عينات عشوائية من أشخاص أصحاء كمجموعة سيطرة.

وجدت هذه الدراسة إن الفئة العمرية من ٥٠سنة إلى ٦٠ سنة هم الأكثر تقبلا لحدوث الجلطة بنسبة ٤٥% وان حدوثها كان أكثر في الرجال بنسبة ٥٥% بينما في النساء ٤٥% وبدرجة حرية (P٠.٠١).

تم استخلاص الحامض النووي الرايبوزي منقوص الأوكسجين من عينات الدم موضع الدراسة واجري تحليل التروبونين للتحري عن حالات حدوث الجلطة .

تم تضخيم الدنا المستخلص من عينات المرضى باستخدام جهاز التضخيم التسلسلي العشوائي وباستخدام إحدى عشر بادئ متخصص لجين F٥ وهي البادئ الأول (Fve٣) وبحجم (٢٢٨bp) ، البادئ الثاني (Fve٤)وبحجم(٣١٠bp)، البادي الثالث (Fve٦) وبحجم (٥٤٧bp)، البادئ الرابع (Fve٧) وبحجم (٢٤١bp)، البادئ الخامس (Fve8) وبحجم (٣٠٦bp)، البادئ السادس (Fve١٢) وبحجم (٢٨٦bp)، البادئ السابع (Fve١٣a) وبحجم (٢٦٠bp)، البادئ الثامن (Fve١٣c) وبحجم (٣١٧bp)، البادئ التاسع (Fve١٥) وبحجم (٦٠٠bp)، البادئ العاشر (Fve١٦) وبحجم (٣٣٣bp)، والبادي الحادي عشر(Fve٢٥) وبحجم (٣٩٠bp).

تم استقراء تتابع سلسلة ألدنا المأخوذ من عملية التضخيم التسلسلي العشوائي لجين F٥ وأظهرت نتائج التحليل للتتابع وجود طفرات نقطية قسمت كمايلي : ٢٤,٨٦% كانت طفرات استبدال و٢٨,٥٧% كانت طفرات إدخال وأخيرا ٢٨,٥٧% كانت طفرات حذف كما تم تشخيص طفرة لـيدن في عدد من المرضى.

جمهورية العراق
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كلية العلوم

الدراسة الجزيئية للجلطة الدموية في عينه من المرضى العراقيين.

رسالة

مقدمة إلى مجلس كليه العلوم- جامعه النهرين كجزء من متطلبات نيل

درجة الماجستير في علوم التقانة الإحيائية

من قبل

مهند نجم عبدالله

بكالوريوس علوم حياة/كلية العلوم /جامعة النهرين/ ٢٠٠٥

بإشراف

د. رحاب صبحي رمضان

أستاذ مساعد

تشرين الأول ٢٠١٥ **ذي الحجة ١٤٣٦**