Sudan University of Science and Technology

College of Graduate Studies

Detection of Plasminogen Activator Inhibitor-1 Gene Mutation 4G/5G among Deep Venous Thrombosis Sudanese Adult Patients in Khartoum State

الكشف عن الطفرة الجينية 5G/4G في مثبط منشط البلازمينوجين1في السودانيين البالغين المرضى بالخثار الوريدية العميقة في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements for the award a degree of M.Sc in Medical Laboratory Sciences (Hematology and Immunohematology).

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March 2019

الآية

قَالَ تَعَالَىٰ:

﴿لَا يُكَلِّفُ ٱللَّهُ نَفْسًا إِلَّا وُسْعَهَا ۚ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا ٱكْتَسَبَتْ ۗ رَبَّنَا لَا تُؤَاخِذْنَآ إِن نَّسِينَآ أَوْ أَخْطَأْنَا ۚ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَآ إِصْرًا كَمَا حَمَلْتَهُۥ عَلَى ٱلَّذِينَ مِن قَبْلِنَا ۚ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِۦ ۖ وَٱعْفُ عَنَّا وَٱغْفِرْ لَنَا وَٱرْحَمْنَآ ۚ أَنتَ مَوْلَىٰنَا فَٱنصُرْنَا عَلَى ٱلْقَوْمِ ٱلْكَٰفِرِينَ ﴿٢٨٦﴾﴾

سورة البقرة الآية[٢٨٦]

Dedication

To soul of my mother

To my beauty family

To my lovely friends

To all teachers that give me a new knowledge to

reach this educational degree

Acknowledgment

Firstly grateful thanks of Allah for helping me to do this work.

Thanks a lot to Prof. Babiker Ahmed Mohammed for good supervising.

Grateful thanks to Ustaz- Abdullah Mousa and Ustaz- Alhaj Nour Alden for helping me to do this study.

Beautiful thanks to all Research lab staff and colleagues for My assistance.

Abstract

A number of prothrombotic and fibrinolytic disorders may lead to venous thromboembolism(VTE). Venous thrombo embolism(VTE) includes deep vein thrombosis (DVT)and pulmonary embolism(PE).The disease is caused by many factors some of which are congenital and others are acquired. A 4G/5G polymorphism located in the promoter region of plasminogen activator inhibitor-1 (PAI-1) gene has been found to be commonly associated with the high levels of PAI-1 and might be a risk factor for deep vein thrombosis (DVT).In this study we aimed to investigate the relationship between genetic mutation in PAI-1 and the association with deep venous thrombosis.

This is a cross sectional study which was conducted in Khartoum state during the period from April 2018 to March 2019. Eighty individuals were included in this study. Forty are represent the patient group. Nine males and Thirty one were females ;median age 36.5 years ranging from 18 to 55 years. The control group (healthy volunteers) included twenty three men and seventeen women; median age 34.5 years; ranging from 18 to 51 years. Among study group 20 %(16/40) were pregnant women, 32.5%(26/40) women tacking contraceptive pills, 5%(4/40) smokers and 6.2 %(5/40) Were obese.

Blood samples were collected under sterile condition. Two milliliter of whole blood poured in ethylene di amine tetra acetic acid (EDTA) anticoagulant container which used for the molecular techniques.

Analysis done in Research lab of Sudan University of Science and Technology, and the genetic mutation detected by using Allele Specific Polymerase Chain Reaction(ASPCR).

The results of genetic analysis showed that: plasminogen activator inhibitor-1(PAI-1) 4G/4G allele had the highest prevalence in the all individuals participate in this study(17(21.2%)) , the 5G/5G , 4G/5G alleles had less prevalence(6(7.5%), 8(10.0%)) respectively. in patient group 10 had the 4G/4G allele, 3 had 5G/5G allele,4 had 4G/5G allele and 23 had wild type allele.

The difference between patient group and control group was statistically insignificant (X^2= 0.713 / *P*=0.870).

In conclusion: The non-significant relation indicated that the investigated polymorphism was not main risk factor four DVT in our population.

Future work should focus on association of other polymorphisms and genes that contributing to DVT.

مستخلص البحث

هناك عدد من الاضطرابات البروتينية والفيبرينية تؤدي إلي الخثرات الدموية الوريدية(VTE). تشمل الخثرات الوريدية الخثرات الوريدية العميقة (DVT)وانسداد الشريان الرئوي (PE). المرض ناتج عن العديد من العوامل بعضها عوامل خلقيه والبعض الآخر عوامل مكتسبة .

وجد أن تعدد الأشكال 4G/5G الموجود في منطقه المروج في جين مثبط منشط البلازمنوجين -1 له غالبا علاقة بارتفاع بمعدل مثبط منشط البلازمنوجين -1 ويمكن أن يكون عامل خطر للإصابة بالخثرات الوريدية العميقة .

في هذه الدراسة عمدنا إلى دراسة العلاقة بين الطفرة الجينية في مثبط منشط البلازمنوجين -1 وعلاقتها بخثرات الأوردة العميقة.

أجريت دراسة الحالات والضوابط المقطعية في ولاية الخرطوم خلال الفترة من ابريل 2018 إلى مارس 2019 .

تضمنت الدراسة عدد 80 شخص , منهم 40 تمثل مجموعه الدراسة أو المرضى وتضم تسعه رجال وواحد وثلاثون امرأة تتراوح أعمارهم بين 18-55 عام بمتوسط عمر 36.5 عام.

اشتملت الدراسة على أربعون شخصا أصحاء وسميت مجموعه الضبط وتضم ثلاثة وعشرون رجلا وسبعة عشر امرأة تتراوح أعمارهم بين 18 -51 عام بمتوسط عمر34.5 عام .

اشتملت مجموعه المرضى على 20% نساء حوامل ,32.5% نساء استخدموا موانع حمل ,5% مدخنين و6.2 % كانوا بدناء .

أخذت عينات دموية في وسط معقم وتم اخذ 2 مل دم وريدي في ماده الاثيلين تنائي رباعي حامض الخليك (EDTA) المضادة للتجلط التي استخدمت للتقنيات الجزيئية .

اجري التحليل المعملي في معمل الأبحاث بجامعة السودان للعلوم والتكنولوجيا, ولفحص الطفرات الجينية استخدمنا تقنيه تفاعل البوليمريز المتسلسل محدد الأليل (ASPCR).

أظهرت نتائج التحليل الجيني :أن معدل انتشار مثبط منشط البلازمنوجين-1 النمط 4G/4G كانت الأعلى في كل الأشخاص المشتركين في هذه الدراسة (17(21.2)) , النمط 5G/5G و النمط 4G/5G لديهم معدل الانتشار الأقل (6(7.5%)),(8(10.0%)) على التوالي.

في مجموعة المرضى وجد أن 10 من المرضى يحملون النمط 4G/4G, ثلاثة يحملون النمط 5G/5G, 4 يحملون النمط 4G/5G و23 يحملون النمط الطبيعي.

لم يكن الفرق بين مجموعة المرضي ومجموعة الضبط ذا دلالة إحصائية(X^2= 0.713 / P=0.870).

يستنتج من هذه الدراسة ان العلاقة غير الجوهرية بين أشكال الطفرة الوراثية التي تم فحصها ومرض خثرات الأوردة العميقة ليست عامل خطر أساسي للإصابة بخثرات الأوردة العميقة في مجموعة البحث.

يجب أن يركز العمل المستقبلي على الارتباط بين تعدد الأشكال والجينات الأخرى التي يمكن ان تسهم في تخثر الأوردة العميقة.

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List of abbreviations:

acas: anti cardiolipin antibodies

AT III: Antithrombin III

BP: Base Pair

CI: Chi Squire

DNA: Deoxy Ribo Nucleic Acid

DVT: Deep Vein Thrombosis

EDTA: Ethylene Di Amine Tetra Acetic acid

FVL: Factor V Laden

HMW: High Molecular Weight

KDa: Kilo Dalton

KHCO3: Potassium bicarbonate

La: Lupus antibody

ml: Mil litter

NH4CL: Ammonium chloride

OR: Odd Ratio

PAI-1: Plasminogen Activator Inhibitor -1

PCR: Polymerase chain Reaction

PCR-ARNS: Polymerase Chain Reaction- Amplification Refractory Mutation System

PE: Pulmonary embolism

Plg: Plasminogen

Pn: Plasmin

RNA: Ribo Nucleic Acid

RPM: Round Per Minute

SAK: Staphylokinase

SDS: Sodium Dodocyl Sulfate

SK: streptokinase

SNPs: Single Nucleotide Polymorphisms

SPSS: Statistical Package for the Social Sciences

TF: Tissue Factor

tPA: tissue Plasminogen Activator

TXA2: Thromboxane A2

UPA: Uro kinase-type Plasminogen Activator

UPAR: Urokinase –type Plasminogen A

VTE: Venous Thromboembolism

VWF: Von Willebrand Factor

WBC: White Blood Cells

µL: micro litter

Chapter one

Introduction and literature review

1.1 Introduction:

The processes of blood coagulation(homeostasis)and clot dissolution (fibrinolysis) are intricate and interrelated (Kern, 2002).

Homeostasis' is process by which hemorrhage is arrested(Bain *et al*., 2003).

The maintenance of circulatory hemostasis is achieved through the process of balancing bleeding (hemorrhage) and clotting (thrombosis)(Turgeon, 2012).

The hemostatic system consist of blood vessels, platelets, and the plasma coagulation system including the fibrinolytic factors and inhibitors(Munker *et al*., 2007).

Hemostasis consists of three steps: vasoconstriction, which is media by reflex neurogenic mechanisms; platelet plug (primary hemostasis); and activation of the coagulation cascade (secondary hemostasis) (wahed and Disgupta, 2015).

Genetic and acquired influences on the platelets and vessel wall as well as on the coagulation and fibrinolytic system, determine whether normal hemostasis or bleeding or clotting symptoms will result(Longo *et al*., 2011).

Thrombophilia is heritable or acquired disorder of haemostatic mechanism predisposing to thrombosis, typically venous, Arterial thrombosis is usually the result of atherosclerosis not blood hypercoagulability (Provan *et al*., 2004).

Suggestions of an inherited thrombophilia include thrombosis without any predisposing condition(no surgery, injury, prolonged inactivity),

thrombosis at a young age(less than about 40 to 45),thrombosis in unusual site(upper extremities, mesenteric vessels, hepatic or portal veins, cerebral veins), and a family history of thrombosis(Kern, 2002).

Thrombosis is multifaceted disorder resulting from abnormalities in blood flow, coagulation system, platelet function, leukocyte activation molecules, and the blood vessels; It is the inappropriate formation of platelets or fibrin clot that obstruct blood vessels(Keohane *et al*., 2016).

The theoretical causes of thrombophilia are physical, chemical or biological events; inappropriate and uncontrolled platelet activation; uncontrolled blood coagulation system inactivation and uncontrolled fibrinolysis suppression(Keohane *et al*., 2016).

The balance of hemostasis is aided by those products that restrain fibrinolytic activity; These products, plasminogen activator inhibitor 1 (PAI-1) and alpha-2-antiplasmin, act upon different substrates in the fibrinolytic system(Ciesla, 2007).

When PAI-1 is functionally or constituently deficient, bleeding may occur due to inadequately suppressed fibrinolysis ,when there is excess PAI-1, the fibrinolytic capacity is decreased, leaving the patient at increased risk of thrombosis(Bern and McCarthy, 2010).

Recently, a common functional deletion/insertion polymorphism (4G/5G) in the promoter of the PAI-1gene located 675 bp upstream from the transcription start site was reported to result in the elevated expression of PAI-1 gene (Balta *et al*., 2002).

A number of prothrombotic and fibrinolytic disorders may lead to venous thrombosis. One of the most common genetic disorders for deep vein thrombosis (DVT) is factor V Leiden (FVL). HR2 haplotype mutation has also been seen in activated protein C resistant patients(Akhter *et al*., 2010).

An impaired fibrinolytic system may lead to DVT and the impairments occur due to mainly 2 reasons low concentration of tissue plasminogen activator (tPA) or increased concentration of its inhibitor, plasminogen activator inhibitor-1 (PAI-1), in plasma(Akhter *et al*., 2010).

The aim of this study was to investigate whether the 4G/5G polymorphism of plasminogen activator inhibitor-1 (PAI-1) is associated with increased risk for deep venous thrombosis in Sudanese population.

1.2 Literature review

1.2.1 Hemostasis:

Haemostasis is one of a number of protective processes that have evolved in order to maintain a stable physiology, 'Haemostasis' refers more widely to the process whereby blood coagulation is initiated and terminated in a tightly regulated fashion, together with the removal (or fibrinolysis) of the clot as part of vascular remodeling(Hoffbrand and Moss, 2016).

1.2.1.1 Primary hemostasis:

Primary haemostasis results from complex interactions between platelets, vessel wall and adhesive proteins leading to the formation of initial 'platelet plug'; The endothelial cells lining the vascular wall exhibit the antithrombotic properties due to multiple factors: negatively charged heparin like glycosamino glycans, neutral phospholipids, synthesis and secretion of platelet inhibitors, coagulation inhibitors and fibrinolysis activators, In contrast, sub endothelial layer is highly thrombogenic and contains collagen, Von Willebrand factor(vWF) and other proteins like laminin, thrombospondin and vitronectin that are involved in platelet adhesion. Any vascular insult results in arteriolar vasospasm, mediated by reflex neurogenic mechanisms and release of local mediators like endothelin and platelet derived thromboxane A2(TxA2)(Palta *et al* .,2014).

1.2.1.2 Secondary hemostasis (Coagulation pathway):

Thrombin is the key effecter enzyme of the coagulation system, having many biologically important functions such as the activation of platelets, conversion of fibrinogen to a fibrin network, and feedback amplification of coagulation, The precise and balanced generation of thrombin at sites

of vascular injury is the result of an ordered series of reactions collectively referred to as blood coagulation, the system is triggered on the surface of extra vascular cells by the exposure of tissue factor to blood (Dahlback *et al*., 2000).

1.2.1.2.1 Extrinsic pathway:

It is considered as the first step in plasma mediated haemostasis. It is activated by TF, which is expressed in the sub endothelial tissue. Under normal physiological conditions, normal vascular endothelium minimizes contact between TF and plasma procoagulants, but vascular insult expose TF which binds with factor VIIa and calcium to promote the conversion of factor X to Xa (Palta *et al*., 2014).

1.2.1.2.2 Intrinsic pathway:

It is a parallel pathway for thrombin activation by factor XII. It begins with factor XII, HMW kininogen, prekallekerin and factor XI, which results in activation of factor XI. Activated factor XI further activates factor IX, which then acts with its cofactor(factor VIII) to form tenase complex on a phospholipid surface to activate factor X (Palta *et al*.,2014).

1.2.1.2.3 Common pathway:

Activated factor X along with its cofactor(factor V), tissue phospholipids, platelet phospholipids and calcium forms the prothrombinase complex which converts prothrombin to thrombin. This thrombin further cleaves circulating fibrinogen to insoluble fibrin and activates factor XIII, which covalently cross links fibrin polymers incorporated in the platelet plug. This creates a fibrin network which stabilises the clot and forms a definitive secondary haemostatic plug (Palta *et al*., 2014).

1.2.2 Fibrinolysis:

1.2.2.1 Fibrin formation and clot structure:

Fibrinogen, a soluble 340-kDa protein, circulates in whole blood at concentrations of 2–4 mg/mL It consists of two sets of three distinct disulfide-linked polypeptide chains (Aα, Bβ, and γ), whose synthetic programs are directed by three separate genes on chromosome 4, Thrombin's major molecular target is fibrinogen, which is converted to fibrin monomers as thrombin removes N-terminal fibrinopeptides A and B, The resulting monomer is a disulfide-linked trinodular protein whose N- and C-termini converge at the E- and D-nodules, respectively(Chapin and Hajjar, 2015).

Assembly of fibrin fibers then proceeds in a stepwise fashion. After an initial lag phase, release of fibrinopeptide A encourages protofibril formation by the lateral aggregation of fibrin fibers, wherein the E domain of one homodimer interacts with the D domain of a second to generate a half-staggered, overlapping fibrillar pattern within the developing thrombus Fibrin is cross-linked at lysine residues by factor XIIIa and forms fibrillar aggregates, which, together with platelets and red blood cells, provide structural integrity to the growing thrombus (Chapin and Hajjar, 2015).

1.2.2.2 Fibrinolysis mechanism:

Fibrinolysis is the resulting of interactions among multiple plasminogen activators and inhibitors consituting the enzymatic cascade ultimately leading to the degradation of fibrin. A crucial reaction of the plasminogen activator system is the conversion of plasminogen to plasmin by plasminogen activators(Cesari *et al*., 2010).

Pn also cleaves a variety of other substrates including extracellular matrix proteins, and activates other proteases and growth factors(Weisel and Litvinov, 2014).

Under normal conditions, fibrinolysis, i.e. the process in which an intravascular thrombus is dissolved under the influence of proteolytic enzymes, takes place mainly on the surface of, for example, endothelial cells thanks to appropriate cellular receptors; These receptors bind plasminogen, uPA, and annexin II, which may form a complex with plasminogen and tPA. Proteolytic degeneration of the fibrin network takes place in the blood only under pathological conditions(Dubis and Witkiewicz, 2010).

1.2.3 Plasminogen:

Plasminogen is primarily present in the plasma, and the liver represents its primary site of synthesis (Cesari *et al*., 2010).

1.2.3.1 Plasmenogen gene structure and expression:

The plasminogen gene is located on the long arm of chromosome 6 at band q26-q27 It spans 52.5 kb of DNA with 19 exons; It is in close proximity to two genes for apo lipoprotein A and for the plasminogen-related genes A and B. Plasminogen expression is normally stable, with the regulation Of the activity of the fibrinolytic system occurring mainly via the Regulation of the plasminogen activators and their inhibitors(Greer *et al*., 2014).

1.2.4 Plasminogen activators:

The activators of plasminogen (Plg) are the serine proteases t-PA or u-PA and bacterial proteins that acquire proteolytic activity after the interaction with human Plg or Pn, streptokinase (SK), and staphylokinase (SAK). tPA and SAK are fibrin-selective, remaining bound to fibrin and protected from rapid inhibition, while SK and two-chain u-PA are non-

fibrin-selective enzymes, activating both Plg in the circulating blood and fibrin-bound Plg(Weisel and Litvinov, 2014).

1.2.4.1The role of plasminogen activator system:

The plasminogen activator system plays a key role in a wide range of physiological and pathological processes, including coagulation, fibrinolysis, inflammation, wound healing, and malignancy(Cesari *et al*., 2010).

1.2.5 Regulation of fibrinolysis:

Regulation of fibrinolysis occurs at least at three levels: (1) by inhibition of proteolytic enzymes responsible for activation of plasminogen eg, plasminogen activator inhibitors (plasminogen activator inhibitor [PAI], (2) by inhibition of plasmin eg,2-antiplasmin,and (3) by thrombin, in response to activation of the coagulation cascade. Clots with a fibrinolysis-resistant architecture are formed when the initial rate of thrombin formation is relatively high (Mosnier and Bouma, 2006).

1.2.6 Plasminogen activator inhibitors:

Plasminogen activator inhibitors (PAIs)are classified as a subgroup of the serine protease inhibitor (serpin) super-family with a common characteristic of possessing an arginine in the reactive center, The members, including PAI-1, PAI-2, PAI-3 (protein C inactivator), and protease nexin 1 (PN-1), act as the inhibitors of tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) and thrombin (Huang and Lee, 2005).

1.2.6.1 Plasminogen activator inhibitor-1 (PAI-1):

Plasminogen activator inhibitor-1 (PAI-1) is an acute phase protein that is usually only expressed in a dipocytes and endothelial cells, but it can be highly expressed by most cells in response to stress (Incalcaterra *et al*., 2014).

1.2.6.2 Plasminogen activator inhibitor-1 gene structure and expression:

The human gene for PAI-1 is located on chromosome 7 bands q21.3-q22 in close proximity to the loci for erythropoietin, paraoxonase, and cystic fibrosis; It covers 12.2 kb of DNA with nine exons specifying the 23 amino acids of the signal peptide and 379 amino acids of the mature protein. Introns, totaling approximately 9,000 bp, define boundaries of individual structural subdomains or are found in random coil regions of the protein(Greer *et al*.,2014).

1.2.6.3 PAI-1 is a regulator of PA system:

PAI-1 is an important regulatory protein involved in the proteolytic and fibrinolytic pathways of plasminogen activator. It is the major inhibitor of two types of plasminogen activator, i.e. tPA and uPA.,tPA binds to fibrin and convert plasminogen to plasmin in the clot and is known as the primary fibrinolytic activator ,Distinctly, uPA has very low affinity for fibrin, but it is the major PA form expressed by the migrating cells and its activity is mediated by uPA receptor (uPAR). uPA binds to uPAR on cell surface to initiate a proteinase activity, which in turn leads to the activation of plasmin ,Plasmin is a proteinase exhibiting a wide range of substrate spectrum(Huang and Lee, 2005).

1.2.7 Control of coagulation:

A disrupted balance between procoagulant and anticoagulant elements of coagulation can lead to prothrombotic events and associated morbidity and mortality. Thromboses can be induced by the excess function of procoagulant factors or by the failure of anticoagulant proteins to suppress coagulation response. These conditions may result from physiological changes (e.g., aging), hereditary causes, acquired disease state (e.g., atheromatous disease, cancer, anti phospholipid syndrome),

drugs (e.g., oral contraceptive), and iatrogenic causes (e.g., drug-elutingstent) (Tanaka *et al*., 2009).

1.2.8 Coagulation regulation:

These done by factors that inhibit the coagulation cascade and ensure the action of thrombin is limited to the site of injury(Mehta, 2000).

1.2.8.1 Coagulation regulatory factors:.

Antithrombin inactivates serine proteases, principally factor Xa and thrombin; a-2 macroglobulins,a-2 antiplasmin, a-2 antitrypsin and heparin cofactor II also inhibit serine proteases; protein C and protein S are vitamin k-dependent proteins made in the liver, which inhibit the coagulation by inactivating factor Va and VIIa ,also enhance fibrinolysis by inactivate the TPA inhibitor ;Tissue factor pathway inhibitor inhibits the main in vivo coagulation pathway by inhibiting factor VIIa and factor Xa(Mehta, 2000).

1**.2.9 Coagulation disorders:** .

Haemophilia is the name given to an increased bleeding tendency; It can be heritable or acquired. The commonest heritable bleeding disorder is mild von Willebrand disease, affecting 1 per few hundred of the populaion. This heritable disorder is not typically referred to as haemophilia but in the broadest sense of the definition it is a form of haemophilia. 'Classical haemophilia', also termed 'haemophilia A', is due to factor VIII deficiency and affects only 1 per 10,000 male births. It is therefore encountered infrequently in non-haematological practice. The most common acquired form of haemophilia is that due to oral anticoagulant therapy as 1 per 100 of the population of many countries are now taking long-term warfarin or similar anticoagulants(Provan *et al*.,2004).

Conversely, thrombophilia is used to describe an increased tendency to

thrombosis. This can also be heritable or acquired. Heritable thrombophilic defects are often insufficient on their own to cause thrombosis and an additional acquired factor, such as surgery, is often the trigger for an acute thrombotic event(Provan *et al*., 2004).

1.2.10 Thrombosis:

Thrombosis is the pathological process whereby platelets and fibrin interact with vessel wall to fold form a haemostatic plug to cause vascular obstruction(Mehta and Hoffbrand, 2000).

1.2.10.1 Arterial thrombosis:

This occurs in relation to damaged endothelium, exposed collagen and released tissue factor cause platelet aggregation and fibrin formation(Mehta and Hoffbrand, 2000).

1.2.10.2 Venous thrombosis:

Factors affected blood flow (stasis, obesity) alterations in blood constituents and damage to vascular endothelium (caused by sepsis ,surgery or indwelling catheters) are important factors(Mehta and Hoffbrand, 2000).

1.2.11 Thrombophilia:

The term thrombophilia is used to describe inherited or acquired disorders of the haemostatic mechanism that predispose to thrombosis(Hoffbrand and Moss, 2016).

Thrombophilia may be inherited or acquired, and the hypercoagulability state may arise from an excess or hyper function of a procoagulant or a deficiency of an anticoagulant moiety(Wahed and Disgupta, 2015).

1.2.11.1 Acquired causes of thrombophilia:

Acquired causes of thrombophilia are more common that inherited causes; Trauma, surgery, immobilization, pregnancy, hormone replacement therapy, Use of oral contraceptives, paroxysmal nocturnal

hemoglobinuria, etc, However, lupus anticoagulant (lupus anti-body (la)) and anticardiolipin antibodies (acas) are commonly encountered in patients with a higher risk of thrombotic event,the use of oral contraceptives increases the risk of venous thromboembolism as well as arterial thrombosis(Wahed and Disgupta, 2015).

1.2.11.2 Inherited causes of thrombophilia:

genetic factors associated with thrombophilia include factor V Leiden, prothrombin gene mutation, protein C or S defi-ciency, and antithrombin III (AT III) deficiency, whereas rare genetic defects such as hyperhomocysteinemia and dysfibrinogenemia are also established

causes of thrombophilia, Intermediate genetic factors related to thrombophilia include elevated coagulation factors such as elevated factor VIII activity. Elevated activities of factors IX and XI may also be associated with thrombophilia (Wahed and Disgupta, 2015).

1.2.12 venous thromboembolism:

Blood clots in veins usually occur in the legs (a condition referred to as deep vein thrombosis or DVT) or the lungs (pulmonary embolism or PE)(Lim and Moll, 2015).

venous thromboembolism (VTE) is a relatively common and potentially life-threatening condition that affects approximately 100 persons per 100,000 per year in the United States , about one third of patients with VTE present with features of pulmonary embolism (PE) and two thirds present with features of deep venous thrombosis (DVT)(Laine and Goodacre, 2008).

Deep venous thromboembolism (DVT) is a major health problem as a result of thrombosis in the deepvenous of the legs ;The annual incidence of DVT has been estimated between 0.1 and 0.2% of the adult population

DVT is a complicated and multi-factorial disorder which can becaused by acquired and genetic risk factors(Farajzadeh, *et al*., 2014).

1.2.12.1 Risk factors for VTE:

Venous thromboembolism may be provoked by transient and reversible clinical risk factors such as surgery or oestrogen exposure, or long term and permanent factors, such as hemiparesis from stroke ;25% of cases, no clinical cause can be ascertained (idiopathic VtE).About 40–60% of VtE patients in caucasian cohorts have thrombophilia – a haemostatic disorder resulting in a thrombotic tendency.this may be heritable (eg. factor V leiden, prothrombin gene mutation and deficiencies of protein c, protein s and antithrombin),or acquired (eg. antiphospholipid antibodies)(Ho, 2010).

High levels of PAI-1 due to the presence of 4G/4G may contribute to the risk for deep vein thrombosis (DVT) and myocardial infarction, The PAI-1 overexpression and impaired fibrinolysis may also cause compromised and insufficient trophoblast invasion, leading to abnormal placental formation and increased risk ofFetal loss is a common health problem affecting1–5% of women of reproductive age (Dordevic *et al* .,2014).

1.2.12.2 Pathogenesis of DVT:

DVT is a multi-factorial disorder which may occur following a combination of some acquired, and inherited risk factors (Hosseini *et al*., 2015).

Virchow's Triad, first described in 1856, implicates three contributing factors in the formation of thrombosis: venous stasis, vascular injury, and hypercoagulability; Venous stasis is the most consequential of the three factors, but stasis alone appears to be insufficient to cause thrombus formation however, the concurrent presence of venous stasis and vascular injury or hypercoagulability greatly increases the risk for clot formation

The clinical conditions most closely associated with DVT are fundamentally related to the elements of Virchow's Triad; these include surgery or trauma, malignancy, prolonged immobility, pregnancy, congestive heart failure, varicose veins, obesity, advancing age, and a history of DVT (Stone *et al*., 2017).

Venous thrombosis tends to occur in areas with decreased or mechanically altered blood flow such as the pockets adjacent to valves in the deep veins of the leg .While valves help to promote blood flow through the venous circulation, they are also potential locations for venous stasis and hypoxia (Stone *et al*., 2017).

1.2.12.3 Symptoms of DVT:

Pain, swelling, and discoloration due to venous stasis are the most common symptoms of DVT patients. However, several other conditions such as infections, hematoma, joint and/or muscle injuries, and chronic joint conditions may present in the same way(Ending *et al*., 2016). Deep Vein Thrombosis (DVT) of the Leg: Only <30% of patients show classic clinical signs: Calf pain at dorsal flexion of the ankle (Homans' sign) or ballottement of the calf, Tenderness when pressure is applied to the sole of the foot (Payr's sign), Tenderness when pressure is applied along the deep veins of the leg, Dilation of epifascial veins Local swelling, hyperthermia, tightness, fever. (Berger *et al*, 2008).

1.2.12.4 Diagnosis of DVT:

As a consequence, clinical examination alone is insufficient to safely establish or rule out DVT. However, if findings from clinical examination are combined with the assessment of DVT triggers in recent history (such as trauma, surgery, immobilization, long-distance travel, cancer, hormone treatment, and pregnancy), preferably with a standardized scoring system

such as the Wells score a much more accurate assessment of DVT probability is possible(Ending *et al*., 2016).

1.2.12.5 The 4G/5G polymorphism:

The humanPAI-1gene is mapped on chromosome 7q21.3-q22, and several polymorphisms within thePAI-1gene have been described(Su *et al*., 2006).

The 4G/5G polymorphism in the promoter of the PAI-1 gene seems to be one of the DNA sequence variation, which has functional importance in regulating expression of the PAI-1 gene. The 4G/5G polymorphism is located -675 base pairs upstream from the start of transcription site of the PAI-1 gene and is characterized by a single guanosine deletion/insertion, resulting in two alleles containing either 4 or 5 guanosines(G) in a row (Stegnar *et al*.,1998).

Homozygosity for the deletion genotype (4G/4G) has been associated with PAI-1 concentrations higher than those associated with the insertion genotype (5G/5G), and hence with reduced fibrinolytic activity(Glaninger *et al*., 2003).

Angiotensin II plasma levels also influence PAI-1 expression; otherwise it increase the risk of pregnancies, predisposing to prematurity, Intrauterine growth retardation, miscarriage and stillbirth (Babker *et al*., 2015).

In vitro studies suggest that the 4G allele has higher activity than the 5G allele because the 5G allele contains an additional binding site for a DNA-binding protein that could be a transcriptional repressor , It has been reported that the 4G/5G polymorphism could affect a very low-density lipoprotein response element in the PAI-1 promoter sequence and, thus,modulate PAI-1 synthesis in response to hyper triglyceridaemia (Segui *et al*., 2000).

In contrast, a 4G allele binds only a transcriptional activator, resulting in an increased expression of PAI-1 (Dordevic *et al*., 2014).

1.2.12.6 Treatment of DVT:

The goals of treatment for DVT are to stop clot propagation and prevent clot recurrence, PE, and pulmonary hypertension (a potentialcomplication of multiple recurrent PEs). These goals usually are achieved with anticoagulation using heparin followed by warfarin (Coumadin); Despite some controversy about the need to treat isolated calf-vein DVT, a recent evidence-based guideline on antithrombotic therapy recommends at least six to 12 weeks of anticoagulation(Ramzi and Leeper, 2004).

1.2.12.7 Prevention from DVT:

DVT prophylaxis is effective when applied to cases that benefit the most from the substantially reduced thrombotic risk(Pop *et al*, 2014).

The simplest approach to prophylaxis for venous thromboembolism is low-dose unfractionated heparin, 5,000 units administered subcutaneously every eight or 12 hours. However, LMW heparin has been shown to be as effective as unfractionated heparin for surgical prophylaxis of DVT over periods of seven to 10 days (with a possible dose-dependent advantage on bleeding complications) and appears to be at least as effective as warfarin in most postoperative settings(Ramzi and Leeper, 2004).

1.2.13 Previous studies:

In study done in Egypt by Hasan, N.S, 2006, results showed that there was an association between the 4G allele of PAI-1 gene and risk factor of thromboembolism in patients suffering from DVT compared with healthy control(41.7% vs 20%, P= 0.04, OR=285; 95%, CI= 1.089- 7.493).the prevalence of 5G homozygous carriers was significantly lower in patients

than in controls(12.5% vs 40%; p= 0.006,OR= 0.214,95%, CI= 0.074-0.621).

A study done in Europe by Stengar *et al*, 1998 showed that: a single nucleotide insertion/deletion (4G/5G) polymorphism in the promoter region of the PAI-1 gene and metabolic factors were studied in 158 unrelated patients below the age of 61 years (43 ± 11 years, mean ± standard deviation) with history of objectively confirmed venous thromboembolism and in 145 apparently healthy controls.

Patients differed significantly from healthy controls neither in the frequency of the 4G and 5G alleles (0.57/0.43 inpatients and 0.52/0.48 in controls) nor in the distribution of the 4G/5G genotypes. Possession of the 4G/4G or the 4G/5G genotype did not increase relative risk for venous thromboembolic disease and the distribution of the 4G/5G genotypes was neither associated with recurrent nor with spontaneous disease.

Another study done in India by Akhter *et al*, 2010 showed that :

Genotyping for 5G/4G polymorphism showed a prevalence rate of 48 (44%)and 29 (26%) for the 4G/4G genotype in patients and control population, respectively, which is significantly different (P=.0013, x^2=10.303; odds ratio[OR]=3.75). The allelic frequency of 4G allele was150 (68%) and 114 (52%) in patients and control population, respectively, which is also significantly different (P=.0004,x^2=12.273; OR=1.99). These results show that 4G allele is highly significantly associated with DVT patients.

Study done in Iranian population by Farajzadeh *et al*, 2014 the results showed that the genotype distribution for PAI-1 4G/5G polymorphisms in DVT patients were significantly higher than healthy control (P<0.05).

Study done in British by Visanji *et al*,2000 showed that: the 4G allele was more prevalent among cases than among healthy subjects(x^2=8.00, P=0.005) and the odds ratio (OR) for thrombosis associated with either heterozygosity or homozygosity for the 4G allele was 2.43 (P=0.011). Study done in Spain by Segui *et al*, 2000 found that there was no differences between the 4G/5G allele distribution in the DVT group (0.43/0.57) and in the control group (0.42/0.58) were observed. However, the presence of the 4G allele significantly increased the risk of thrombosis in patients with other thrombophilic defects .In the DVT group, a slight, but not significant, difference was observed between this 4G/5G allele distribution in patients with thrombotic recurrence (0.46/0.54) vs. patients with a single thrombotic event (0.42/0.58).

Another study done in Turkey by Kaya *et al*, 2013 showed that: there were no significant statistical differences for PAI-1 4G/5G polymorphism among patient and control groups (p> 0.05).

In study done in Italy by Sartori *et al*, 2003 in 67patients with idiopathic DVT and 98 normal subjects ,By comparison with controls, asignificantlyhigherprevalenceof4G/4G genotype was seen in idiopathic DVT and in thrombophilia patients, although in this latter group the difference only remained significant in cases symptomatic for thrombosis(p=0.01).The 4G/4G genotype was associated with a greater risk of thrombosis both in symptomatic thrombophilia patients(OR 2.85, 95% CI 1.26-6.46) and in idiopathic DVT patients(OR 3.1, 95%CI 1.26-7.59).The greater frequency of 4G allele in symptomatic thrombophilia patients with respect to controls was statistically significant(p=0.04).

In study done in South African urban and rural black participants by Lange *et al*, 2013; the study were cross sectionally analysed. The 5G allele frequency of the 4G/5G polymorphism was 0.85. PAI-1 act

increased across genotypes in the urban subgroup ($p = 0.009$) but not significantly in the rural subgroup.

In 2010 study done in Gaza Strip, Palestine by Al sallout, R.J and Sharif , F.A, the results showed that: there was no significant difference detected in the distribution of polymorphisms for PAI-1,with the 4G/4G genotype present in the study group and in controls ($p = 1.00$).

1.3 Rationale:

Thrombophilia (hypercoagulability or prothrombotic state) is an abnormality of blood coagulation that increases the risk of thrombus formation. Established genetic factors associated with thrombophilia include factor V Leiden, prothrombin gene mutation, protein C or S deficiency, and antithrombin III (AT III) deficiency, gene mutation 4G/5G in PAI-1, which is considered as on cause of DVT. in Sudan studies concerning PAI-1 4G/5G mutation detection are limited; this study is conducted to provide guideline about early detection of DVT patients in Khartoum state and compare it with other studies carried at the same topic. The results of this study may add new records of thrombotic diagnosis of Sudanese DVT patients.

1.4 Objectives:

1.4.1 General Objective:

- To detect Plasminogen activator inhibitor-1 gene mutation 4G/5G among deep venous thrombosis Sudanese Adult patients in Khartoum State.

1.4.2 Specific objectives:

- To detect PAI -1 gene mutation 4G/5G among DVT patients and healthy control group by using allele specific PCR.
- To compare the PCR results of the two groups and get prevalence of mutation for each group.
- To compare this study results with other previous studies done at the same topic.

Chapter two

Material and Method

2.1 Study design:

This study is cross sectional study.

2.2 Study area and duration:

Study carried out in period from April 2018 to March 2019 in order to detect the gene mutation 4G\5G in PAI-1 among deep venous thrombosis Sudanese Adult patients in Khartoum State, Sudan.

2.3 Study population:

The study carried out in 80 Sudanese adults in Khartoum State.

40 patients diagnosed for DVT(cases), thirty one females and nine males; all had been recruited as study group. Males mean age 43 years ranging from 31 to 55 and female mean age 34 years ranging from 18 to 50 years. Forty healthy volunteers were included as control group. They were chosen in accordance to the gender and age of the study group. Both case and control group were unrelated and randomly selected.

2.4 Inclusion criteria:

Confirmed DVT patients were included. The diagnosis done by clinical assessment of risk factors and physical findings followed by Doppler ultrasound confirmation. Forty normal individuals who did not experience any episodes of deep venous thrombosis nor had a history of thrombophilia were included as control group.

2.5 Data collection:

Data was collected by using questionnaire which was specifically designed to obtain information about demographic and clinical data that helped in either including or excluding from the study.

2.6 Sampling technique:

Blood samples were collected under sterile condition and the vein puncture was well dressed. Two milliliter of whole blood poured in ethylene di amine tetra acetic acid (EDTA) container which used for the molecular techniques. EDTA blood were stored at minus seventy degrees Celsius tell the time of examinations.

2.7 Data analysis:

Data was analyzed by using SPSS (version 16), chi squire test.

2.8 Ethical consideration:

The study was done after verbal consent was taken from the individuals under study and informed them with all details about the objectives of the Study.

2.9 Molecular biology:

2.9.1 DNA extraction:

2.9.1.1Reagents:

2.9.1.1.1 Red blood cells lyses buffer:

This solution is composed of: chlorine (NH4CL), potassium bicarbonate (KHCO3) and EDTA 5%, all ingredients dissolved in one liter distilled water and kept at room temperature.

2.9.1.1.2 White cells lysis buffer:

WBC lysis buffer prepared by dissolving four chemicals in 500 distilled water. These chemicals include: Tris-Hcl, EDTA, Nacl and SDS.

2.9.1.1.3 Guanidine hydrochloride:

Strong chaotropic agent used for the denaturation and subsequent refolding of proteins. Prepared by weighing 57.2 grams powder and dissolved in 100 ml distilled water.

2.9.1.1.4 Protienase K:

Proteinase K is commonly used in molecular biology to digest protein and remove contamination from preparations of nucleic acids. Addition of Proteinase K to nucleic acid preparations rapidly inactivates nucleases that might otherwise degrade the DNA or RNA during purification (Crowe et al, 1991).

2.9.1.1.5 Chloroform -20:

Chloroform ensures phase separation of the two liquids because chloroform is miscible with phenol and it has a higher density (1.47 g/cm3) than phenol; it forces a sharper separation of the organic and aqueous phases thereby assisting in the removal of the aqueous phase with minimal cross contamination from the organic phase (Piotr and Sacchi, 2006).

2.9.1.1.6 Ethanol:

DNA is insoluble in alcohols (Ethanol and Isopropanol), therefore 100% alcohol used for the precipitation so we get good amount of DNA. Washing with 70% alcohol is to remove the excess of salts that might have come along with the extraction buffers (i.e. the excess of salts dissolve in the 30% of water).

2.9.1.2 DNA extraction protocol:

The genomic DNA was isolated from peripheral blood leucocytes using Guanidine chloroform Extraction Method. The protocol was performed in three days. First day started with washing whole blood in RBC lysis buffer after thawing at room temperature. Blood placed in sterile falcon tube, lysis buffer added up to the 10 ml mark, tubes centrifuged at 6000 rpm for 15 minutes. After centrifugation supernatant was discarded. These steps were repeated several times until the supernatant became

clear. After that 2ml of WBC lysis buffer, 1ml guanidine, 300 µl ammonium acetate and 25 µl proteinase K were added to the bellet. Last step of day one was the incubation of tubes at 37 ^{0}C for overnight.

On the second day tubes were brought to the room temperature. Two ml of chilled chloroform added to each tube. After that, tubes were agitated in a vortex and centrifuged for 5 minutes at 2500 rpm. Three layers were obtained. The upper layer transferred to a new falcon tube containing 10 ml chilled ethanol previously kept at -20˚c. Then all tubes shaken very well and the second day finalized by placing tubes at -20˚c for overnight.

On day three, tubes were centrifuged and the ethanol discarded, tubes inverted on dry gauze for two hours. When they are dry tubes washed in 70 % ethanol, inverted on gauze until they were dry. After that 100 µl of doubled distilled water added and the mixture was transferred to appindorf tube. Finally, the product kept at -20. (Piotr and Sacchi, 2006).

2.9.2 Mutation detection:

2.9.2.1 Plasminogen activator inhibitor type-1 (PAI-1):

2.9.2.1.1 Primers design and genotypic screening protocol:

Genotypic screening protocols for 4G/5G were done through allele specific PCR. Amplification of genomic DNA was done using the allele-specific primers, insertion 5G allele, deletion 4G allele

each in a separate PCR reaction together with the common downstream primer and a control upstream prime. The control upstream primer is used to verify the occurrence of DNA amplification in the absence of the allele on the genomic DNA(Akhter et al., 2010).

Table(2.1): Primers design:

Primer name	Primer design
insertion 5G allele	5`-GTC TGG ACA CGT GGG GG3`
deletion 4G allele	5`-GTC TGG ACA CGT GGGGA-3`
control upstream primer	5` -AAG CTT TTA CCA TGG TAA CCC CTG GT-3`
common downstream primer	5`-TGC AGC CAG CCA CGT GAT TGT CTA G-3`

(Stegnar *et al*.,1998**).**

2.9.3 Principle of allele specific PCR:

PCR -ARMS is based on the observation that DNA amplification is inefficient or completely refractory if there is a mismatch between the 3 terminal nucleotide of a PCR primer and the corresponding template Taq DNA polymerase lacks a 3 to 5 exonuclease activity, and therefore cannot correct mismatches at the 3 terminus of the primer. As such, complementary base pairing at the 3 end of the primer is required for efficient amplification by Taq DNA polymerase and is a strong determining factor of template specificity. Amplification of the normal allele, and not that of the mutant, is accomplished using a primer that is complementary to the normal allele and has a mismatch between the 3

residue and the mutant allele. Conversely, only the mutant will be amplified if the 3 residue of the primer is complementary to the mutant allele and not the normal allele(Patrinos and Ansorge, 2010).

2.9.4 PCR protocol:

The PCR protocol included initial denaturation step for 3 minutes at 95˚c followed by a 35 cycles: denaturation at 95˚c for 20 seconds, annealing at 55˚c for 10 seconds, extension at 72˚c one for 10 seconds, and a final extension at 72˚c for 3 minutes. The product was detected in 2 % agarose gel electrophoresis.

2.9.5 Detection of PCR product:

The normal gene showed a 257 bp band and the mutant gene yielded a 140 bp for both 5G and 4G polymorph.

Chapter three

Results

3. Results:

Study carried out in period from April 2018 to March 2019 in order to detect the gene mutation 4G\5G in PAI-1 among deep venous thrombosis Sudanese Adult patients in Khartoum State.

This study done in 80 individual, 40 patients diagnosed for DVT(cases), 31 females and 9 males; all had been recruited as study group. Males median age (43) years ranging from (31-55) and female median age (34) years ranging from (18-50) years.

40 healthy volunteers were included as control group, included twenty three men and seventeen women; median age 34.5 years; ranging from 18 to 51 years.

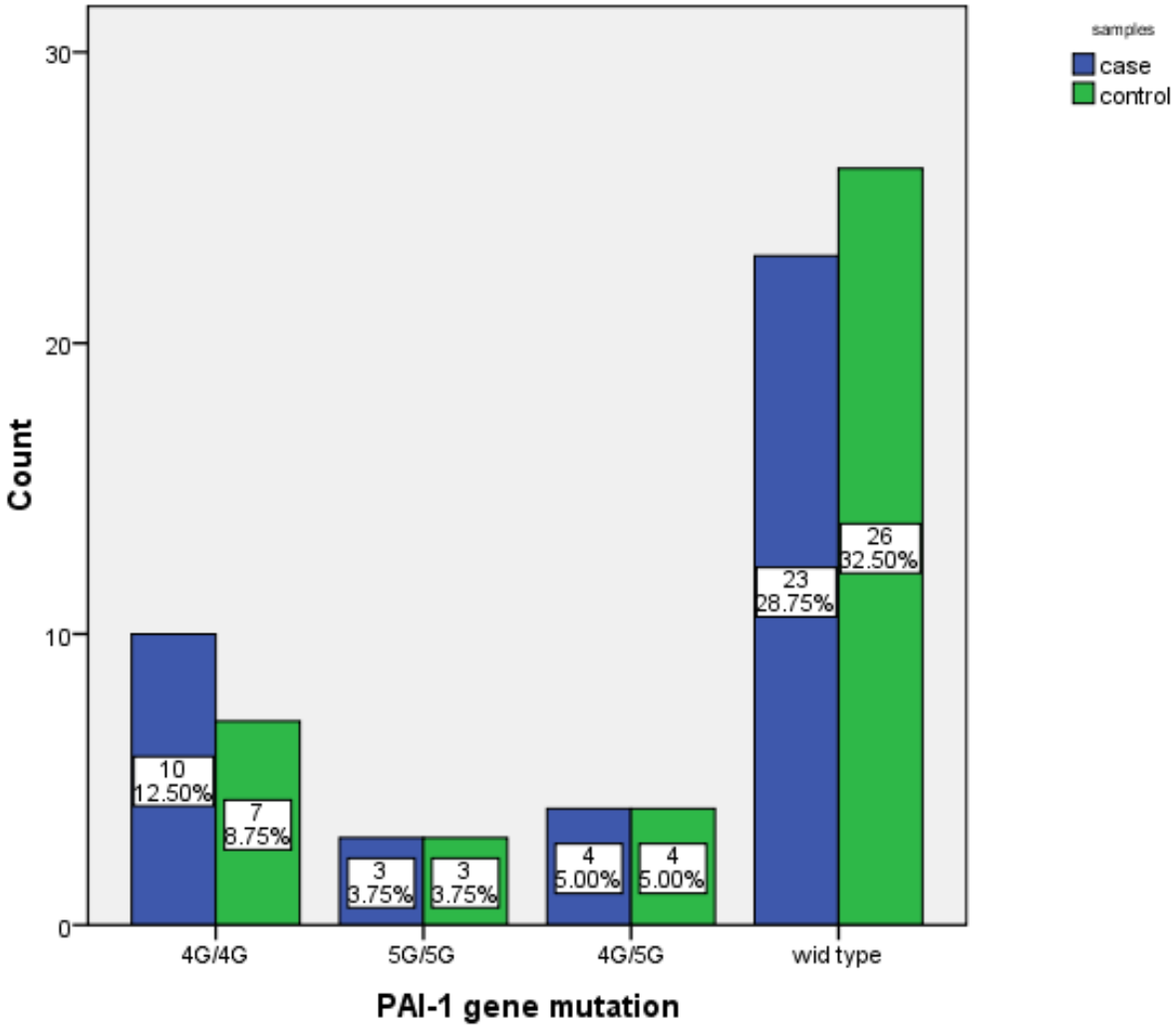
The tables and figures below show the results of this study(frequency , percent, chi- squire and p value).

Table (3.1) Frequency of risk factors among the cases group:

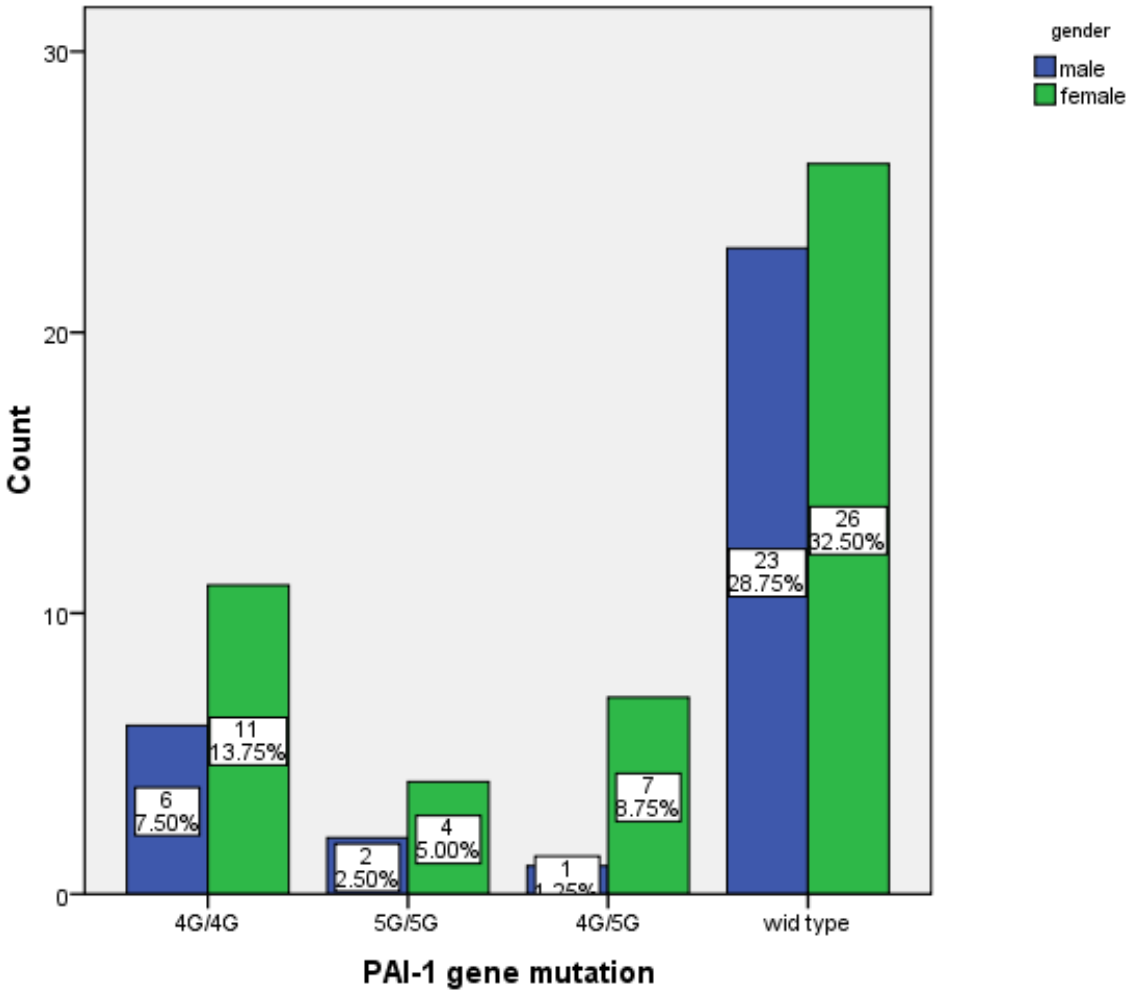
Risk factors	yes N (%)	No N (%)	Total
Pregnancy	(20.0)16	(30.0)24	40
contraceptive	(32.5%)26	(17.5%)14	40
Obesity	5(6.2%)	35(43.8%)	40
Smoking	(5.0%)4	(45.0%)36	40

Table (3.2) Frequency and association of PAI-1 gene mutation among study population:

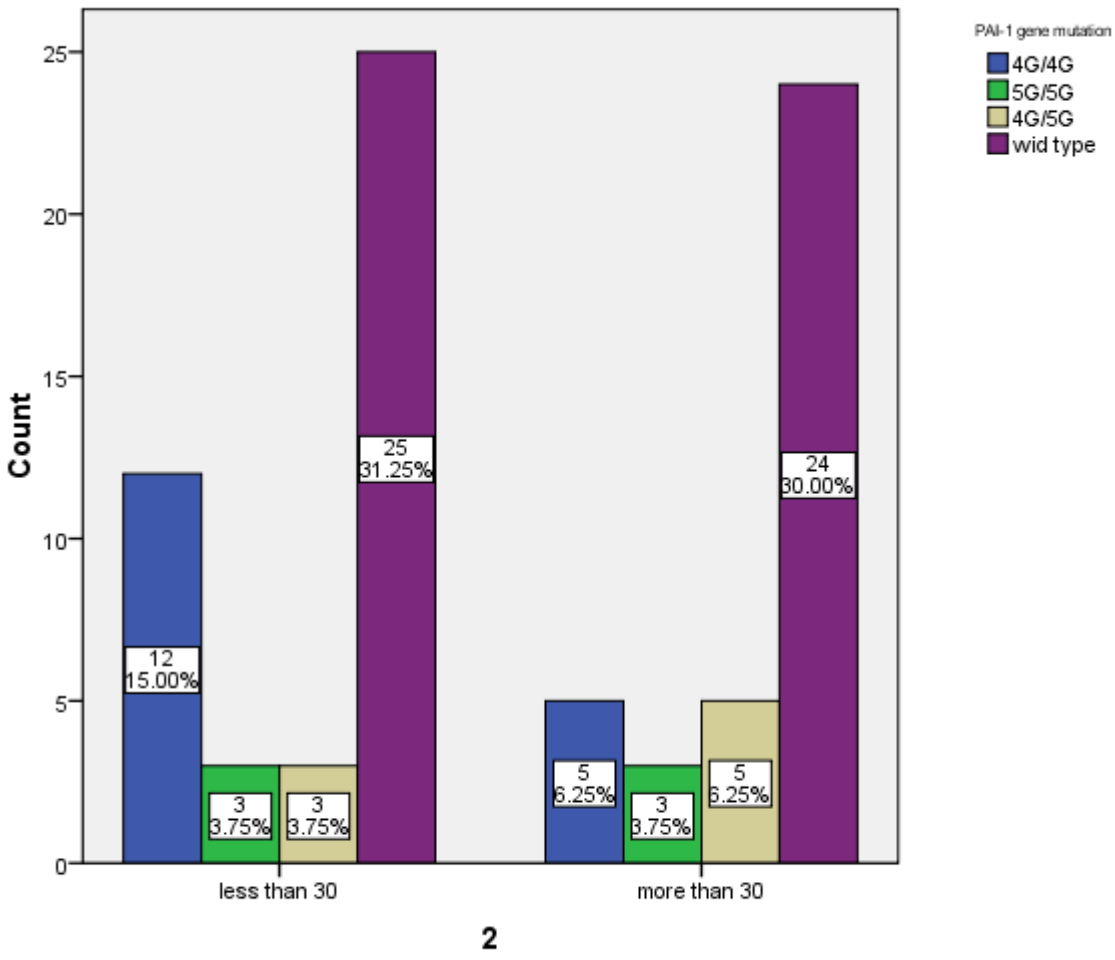
PAI-1 mutation	Samples (count, percent)		Total	Chi-squire value	P value
	Case	Control			
4G/4G	10(12.5%)	8%).7 (8	17 (21.2%)		
5G/5G	3(3.8%)	3 (3.8%)	6 (7.5%)		
4G/5G	4(5.0%)	(5.0%)4	8 (10.0%)	0.713	0.870
Wild type	23(28.8%)	(32.5%)26	49 (61.2%)		
Total	40(50.0%)	40(50.0%)	(100.0%)80		



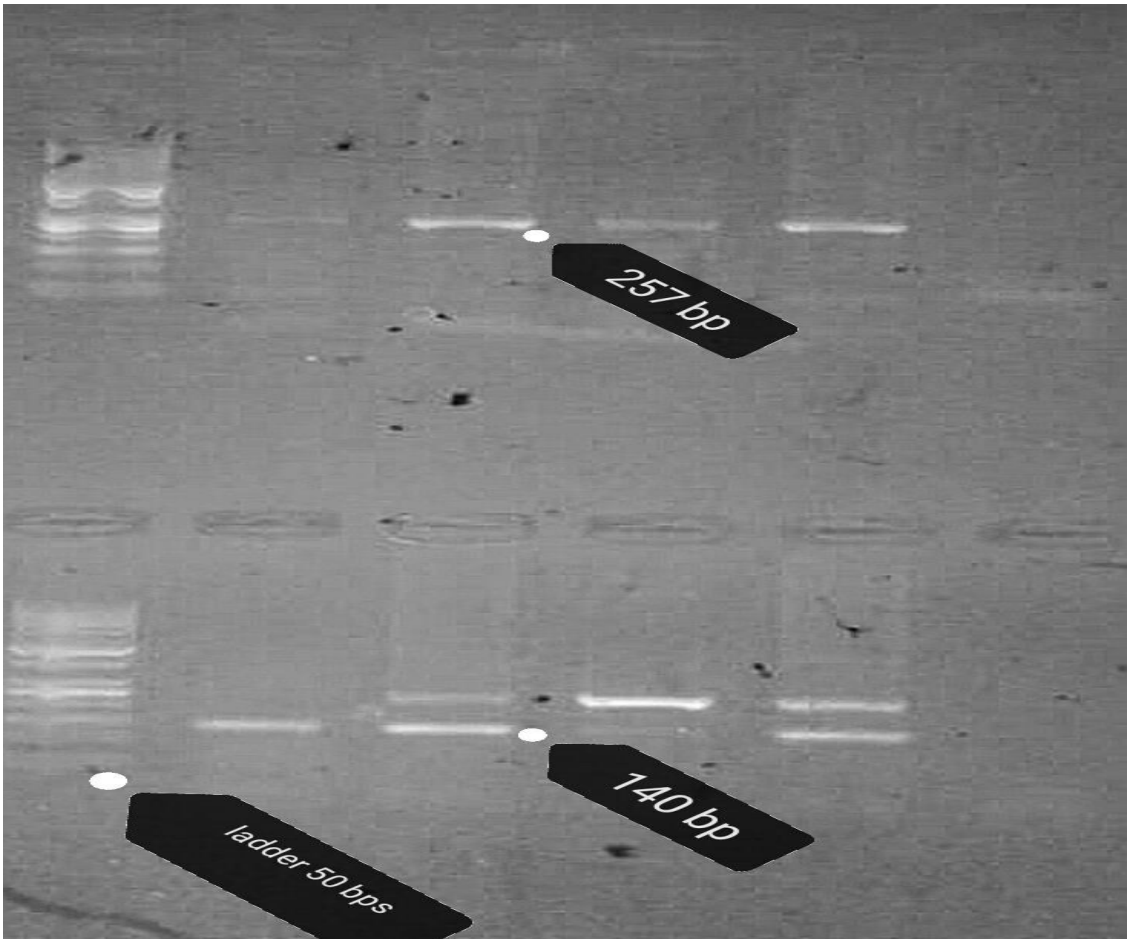
Figure(3.1) The frequency of PAI-1 mutation among cases and control groups



Figure(3.2) Distribution of PAI-1 mutation according to gender in cases and control groups



Figure(3.3) Frequency of PAI-1 among the age groups



Figure(3.4) Ethidium bromide–stained 2%agarose gel showing amplified products of the 4G/5G polymorphism through an allele-specific polymerase chain reaction. Mutant gene 4G/4G, 5G/5G (140 bp), while the wild type gene(257 bp), and the ladder (50 bp).

Chapter four

Discussion, conclusion, and recommendation

4.1 Discussion:

Present study is cross sectional study, carried out in period from April 2018 to March 2019 in order to detect the gene mutation 4G\5G in PAI-1 among deep venous thrombosis Sudanese Adult patients in Khartoum State.

This study carried out in 80 individual, 40 patients diagnosed for DVT(cases), 31 females and 9 males; all had been recruited as study group. Males median age (43) years ranging from (31-55) and female median age (34) years ranging from (18-50) years. The remaining were healthy volunteers as control group, included twenty three men and seventeen women; median age 34.5 years; ranging from 18 to 51 years.

This study have considered at the risk posed by 4G/5G genotype of PAI-1gene. To our knowledge, there are no reports on the prevalence of this polymorphism in Sudanese patients with DVT.

The results of this study found 17 individual had 4G/4G genotype, 6 individual had 5G/5G genotype, 8 individual had 4G/5G and 49 had wild type pattern.

Although the PAI-1 gene mutation appeared in control group and in test group ,but it appeared in the test group due to presence of other risk factors .

In this study there were no significant statisically difference between patient and control groups ($p > 0.05$).this results were confirmed by different authors in other country; Which include Gaza Strip, Palestine (Rami and Fadel , 2010) and Turkey(Kaya *et al*, 2013) ($p > 0.05$).

Otherwise the gene mutation 4G\5G in PAI-1 would reported in many studies in different countries, which give significant results.

For examples study done in Iranian population (Farajzadeh *et al*, 2014) showed that the genotype distribution for PAI-1 4G/5G polymorphisms in DVT patients were significantly higher than healthy control ($P<0.05$). another study in Asian Indian population(Akhter *et al*, 2010) , found high prevalence of 4G/4G and 4G/5G alleles ,patient and control groups were differ significantly ($p < 0.05$). also there was significant increase in 4G/5G genotype in South Africa urban subgroup($p < 0.05$) (Lango *et al*, 2013). While in Italy(Sartori *et al*, 2003) found a significantly high prevalence of 4G/4G genotype ($p < 0.05$). however similar results found in Iranian population (Farajzadeh *et al*, 2014) which showed that the genotype 4G/5G polymorphisms in DVT patients were significantly higher than healthy control ($P<0.05$). also in British (Visanji *et al*,2000) there was significant increase either heterozygosity or homozygosity for the 4G allele ($P<0.05$), and study done in Egypt (Hasan N. S., 2006),showed that the prevalence of 5G homozygous carriers was significantly lower in patients than in controls($p< 0.05$).

4.2 Conclosion:

Study concluded that the PAI-1 gene mutation 4G/5G had non-significant relation , which indicated that the investigated polymorphism was not main risk factor for DVT in our population.

4.3 Recommendations:

- Performing a larger study to investigate the impact of this mutation in other states of Sudan.
- Performing DNA sequencing to know if there is any SNP in other parts of the gene.
- Performing further studies to investigate the relation between other polymorphisms and other genes related to DVT.

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Appendices

Appendix 1: Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Research Title: Detection of Plasminogen activator inhibitor-1 gene mutation 4G/5G among deep venous thrombosis Sudanese Adult patients in Khartoum State

Researcher: Sheima Mohammed Adam
Supervisor: Babiker Ahmed Mohammed

Duration of research:

Name:……. No……

Gender:…….....

Age:…….

Residence: ……..

Mobile number:……..

Clinical and Lab finding at the time of diagnosis:……..

Pregnancy:……...

Contraceptive pills:……

Obesity:……

Smoking:……..

Others (if any):……..

Laboratory results:

PCR:

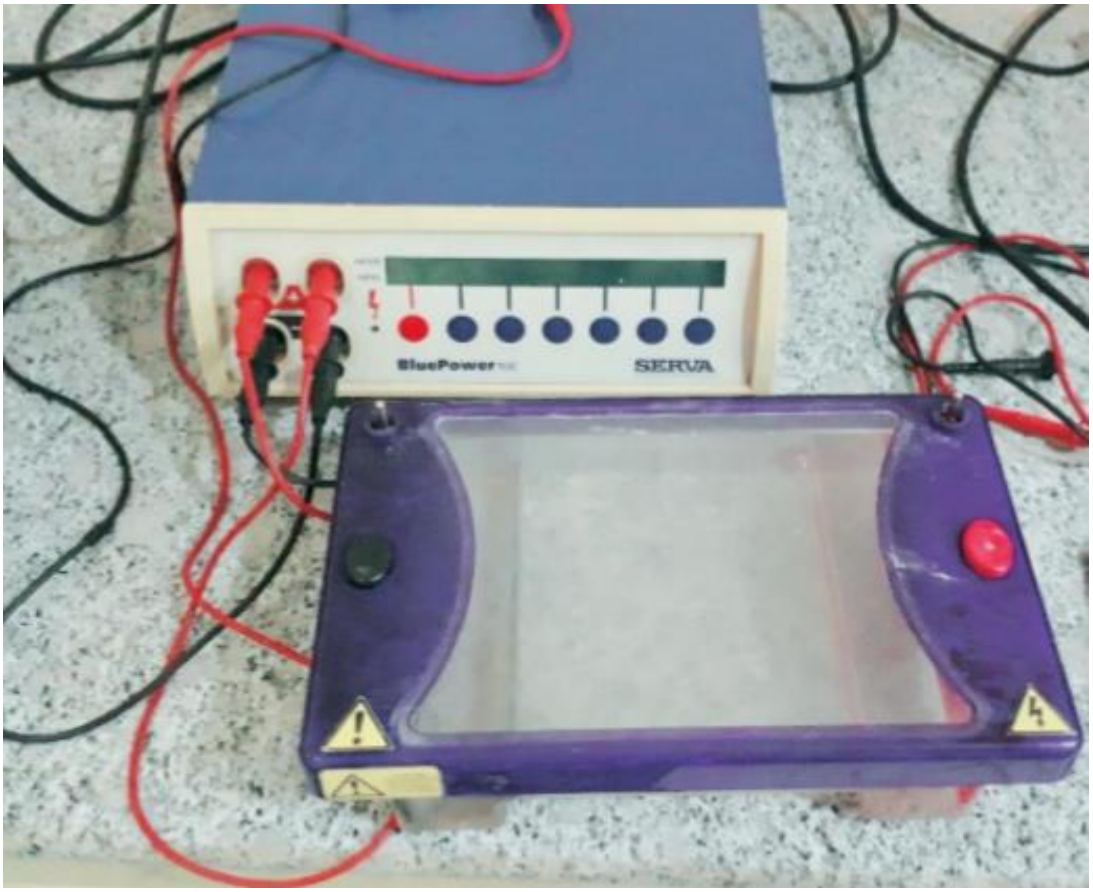
- Plasminogen activator inhibitor -1 4G/5G:……..

Date:…….

Appendix 2

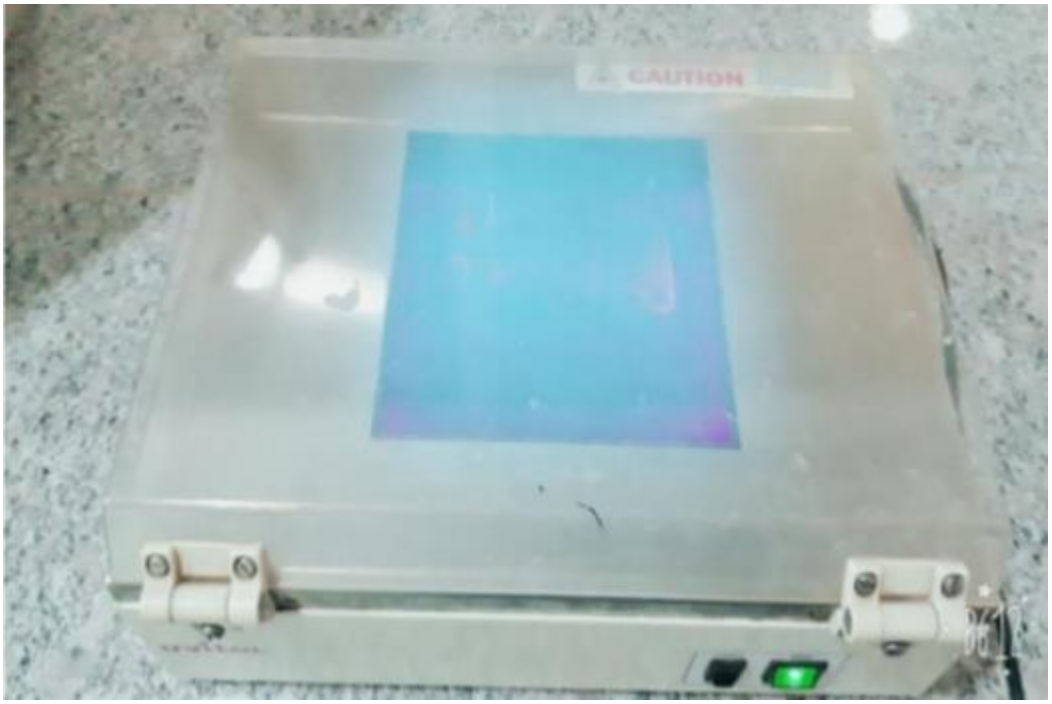
CLASSIC K960 China Thermo cycler Device

Appendix 3



Gel Electrophoresis and Power Supply Devices

Appendix 4



UV Light Transilluminator Device