بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of Higher Education and scientific Research

Shendi University

Faculty of Graduate Studies and Scientific Research

Role of Activated Protein C Resistance in Complicated Pregnancy

A thesis Submitted for partial fulfillment for the Degree of MSc in Medical Laboratory Sciences (Haematology)

By

Sahar Aldaleal Alnagar Yosif
BSc. (Shendi University – 2008)

Supervisor

Dr: Hamza Ahmed Hassan Mohammed
Assistant Profession in hematology medical laboratories science
Shendi University

August-2018

بسم الله الرحمن الرحيم

﴿ وَلَا تُخْزِنِي يَوْمَ يُبْعَثُونَ (٨٧) يَوْمَ لَا يَنْفَعُ مَالٌ وَلَا بَنُونَ (٨٨) إِلَّا مَنْ أَتَى اللَّهَ بِقَلْبٍ سَلِيمٍ (٨٩) ﴾

سـورة الشعـراء

Dedication

To who thought me with love and life

Warm hearted Mam

To endows me kinliness

My great father

To happiness creator

My honey sisters

To who always support me

My dear brothers

To the sources of hopeness

My best friends

Acknowledgement

All thanking and flexing to who help us to complete this study perfectly

Supervisor: Dr: Hamza Ahmed Hassan

Commendations and praises to who aided us in all study steps

Dr: Alfateh M. AbdAllah

Thanks for study colleagues

list of Abbreviations

Abbreviation	Meaning
APC	Activated protein C
APC-R	Activated protein C resistance
APTT	Activated partial thromboplastin time
BMI	Body mass index
FVL	Factor V Leiden
INR	International Normalized Ratio
IUGR	Intrauterine fetal growth restriction
LMWH	Low molecular weight heparin
PAI	Plasminogen activator inhibitor
PTL	Pre term labour
RVV	Rosella viber venom
SGA	Small-for-gestational age
TAT	Thrombin-antithrombin
TM	Thrombomodulin
UFH	Un fraction heparin
VTE	Venous thromboembolism

Abstract

Background:

This is descriptive case control study conducted in Shendi City to evaluate APCR among 34 women with complicated pregnancy in period (Februry2018 to Augest2018)

Thirty four venous blood samples were collected in tri sodium citrate anticoagulant with a ratio (1+9), platelet poor plasma were seprated and freezed at -80°C

The samples were analyzed for APC-R by a functional clotting assay and the data were analyzed by using SPSS and we found there is no homozygous

The wild type (Negative), were represent 73% and heterozygous (carriers) were represent 27%.

Among the carriers group we noticed that 88% were miscarriage and 12% were pre term labour.

All the cases that diagnosed as Stillbirth and pre-eclampsia ladies are wild type

The study also reveals that miscarriage had 73% out of the other types of complicated pregnancy, while PTL, pre-eclampsia and still birth had 9%.

This study conculude that relation between carriers and (Miscariage, PTL).

ملخص البحث

أجريت هذه الدراسة في ٣٤ سيدة في مدينة شندي، في الفترة مابين فبراير٢٠١٨- أغسطس ٢٠١٨م لمعرفة تأثير بروتين سي المثبط على مضاعفات الحمل المتعلقة بالمشيمة. وقد تم جمع البيانات عن طريق الاستبيان.

ثم يتم اخذ عينة من الدم الوريدي على مضاد تجلط سترات الصوديوم بنسبة (٩+١). تم فصل العينة في خلال ساعة من سحبها.تم تجميد العينات ٨٠ درجة تحت الصفر.

لأجراء الاختبار تم تذويب العينة خلال ٥ دقائق في ٣٧ درجة مئوية.استخدمت طريقة حساب زمن التجلط لإجراء الاختبار

تم تحليل البيانات باستخدام برنامج التحليل الإحصائي.

وضحت الدراسة: أن ٧٣% غير مصابين ,٢٧%حاملين لمرض بروتين س المثبط.

٨٨% من حاملي المرض مصابين بالإجهاض و١٢% يولدون مبكراً.

كما وضحت الدراسة أن الإجهاض ٧٣%، الولادة المبكرة٩%، ٩% يموت الجنين قبل الولادة و٩% من السيدات مصابين مقدمات الارتعاج.

List of contents

List of tables

Chapter one

Introduction

Rationale

Objectives

1.1 Introduction

Protein C, a part of the natural anticoagulant system, is a vitamin K-dependent protein zymogen (molecular weight=62,000 da) that is synthesized in the liver and circulates at a plasma concentration of approximately 5 mcg/mL. Protein C is activated to activated protein C (APC) via proteolytic cleavage by thrombin bound to thrombomodulin, an endothelial cell surface membrane protein. [(1)]

APC down regulates the procoagulant system by proteolytically inactivating procoagulant factors Va and VIIIa. Protein S, another vitamin K-dependent coagulation protein, catalyzes APC inactivation of factors Va and VIIIa. APC interacts with and proteolyses factors V/Va and VIII/VIIIa at specific APC binding and cleavage sites, respectively. Resistance to activated protein C (APC resistance) is a term used to describe abnormal resistance of human plasma to the anticoagulant effects of human APC. APC resistance is characterized by a reduced anticoagulant response of patient plasma after adding a standard amount of APC. For this assay, the activated partial thromboplastin time clotting test fails to prolong significantly after the addition of APC [(1)]

The vast majority of individuals with familial APC resistance have a specific point mutation in the procoagulant factor V gene (1691G-A, factor V Leiden) encoding for a glutamine (Q) substitution for arginine (R)-506 in the heavy chain of factor V (factor V R506Q). This amino acid change alters an APC cleavage site on factor V such that factor V/Va is partially resistant to inactivation by APC. Protein C, a part of the natural anticoagulant system, is a vitamin K-dependent protein zymogen (molecular weight=62,000 da) that is synthesized in the liver and circulates at a plasma concentration of approximately 5 mcg/mL. Protein C is activated to activated protein C (APC) via proteolytic cleavage by thrombin bound to thrombomodulin, an endothelial cell surface membrane protein. APC downregulates the procoagulant system by proteolytically inactivating procoagulant factors Va and VIIIa. Protein S, another vitamin K-dependent coagulation protein,

catalyzes APC inactivation of factors Va and VIIIa. APC interacts with and proteolyses factors V/Va and VIII/VIIIa at specific APC binding and cleavage sites, respectively. Resistance to activated protein C (APC resistance) is a term used to describe abnormal resistance of human plasma to the anticoagulant effects of human APC. APC resistance is characterized by a reduced anticoagulant response of patient plasma after adding a standard amount of APC. For this assay, the activated partial thromboplastin time clotting test fails to prolong significantly after the addition of APC.[1]

APC resistance is caused by a single gene mutation in coagulation factor V (FV), which results in the replacement of arginine (R) at position 506 with a glutamine (Q) [FV Q506, FV Leiden (FVL). This replacement eliminates one of the cleavage sites for APC in FV and causes impaired anticoagulation. The resulting hypercoagulable state is a life-long risk factor for venous thrombosis.[2]

However, around 30% of APC-resistant pregnant women have been reported not to be carriers of FVL (here denoted APCR– FVL). APCR FVL [2] has been found to be an independent risk factor for venous thrombosis .This increased thrombosis risk is partly dependent on elevated coagulation FVIII levels. However, even after adjustment for FVIII, APCR FVL– remains to be an independent risk factor [3].

A successful pregnancy outcome is dependent upon adequate uteroplacental circulation, which resembles the venous circulation in terms of its low pressure and low flow velocity.The placental circulation may therefore be particularly susceptible to thrombotic complications in thrombophilic women. [3]

Thrombotic complications at the feto-maternal border may predispose to pregnancy complications such as fetal loss, pre-eclampsia, small-for-gestational age (SGA), abruption of placenta, and preterm delivery. An association has been suggested between the fetal loss and thrombophilias ,caused by protein C ,protein S ,or antithrombin deficiencies. [4,5]

In addition, aconnection between FVL and the occurrence of adverse pregnancy

outcomes has been described Furthermore, carriership of FVL in women has also been associated with a lower volume of blood loss and with lower prevalence of profuse bleeding during delivery, with higher hemoglobin (Hb) values, and with higher ferritin values. The effect of APCR FVL on pregnancy outcomes is not well characterized. [4,5]

In addition, there are no studies reporting on APCRFVL– individuals and delivery-associated blood loss. The purpose of this prospective study was to profile women with APCR FVL– in relation to pregnancy complications and blood loss measurements. [4,5]

1.2 Rationale

Activated Protein C resistance (APCR) is the most frequency thrombophilia that induces thrombotic complications of pregnancy that itself is a hypercoagulable state. Abnormal placental vasculature is the most important mechanism that causes various complications. The screening diagnosis of APCR is influenced by physiological changes of hemostasis in pregnancy and may be difficult. This study aimed to evaluate the risk for thrombosis complications associated with the factor V Leiden mutation in pregnancy, using a lot of women with complications of pregnancy selected from our casuistic. Factor V Leiden mutation and resistance to activated protein C are important risk factors for pregnancy complications. So screening for resistance to activated protein C with FV deficiency plasma is recommended in all pregnant women with placental complications or history of pregnancy complications.

1.3 Objectives

1.3.1 General objective

To detect role of activated protein C resistance in complicated pregnancy

1.3.2 Specific objectives

1. To compare APC-R with miscarriage
2. To know effects of APC-R in women with IUGR
3. Clarification relation between APC-R and PTL
4. To correlate APC-R with still birth
5. To evaluate relationship between genotype and types of pregnancy complication.

Chapter two

Literature review

2. Literature review

2.1 Activated protein C

Protein C is a vitamin K -dependent protein that inhibits blood coagulation. In its activated form (activated protein C, APC), it exerts its inhibitory action by proteolytic cleavage of the procoagulant proteins factor Va and factor VIIIa. Recently, we have described a common mutation in the APC cleavage site of factor V (factor V Leiden) that is associated with APC resistance.' In APC resistance, the patient's plasma does not exhibit the normal anticoagulant response to addition of APC, as reflected in a prolongation of the activated partial thromboplastin time (APTT). In individuals heterozygous for factor V Leiden, the APTT prolongation is moderately decreased, where as in homozygous individuals there is little response at all.' APC resistance is known to be a common and strong risk factor for thrombosis. It is present in 20% of unselected consecutive patients with deep-venous thrombosis, and in 3% of healthy individuals. In individuals from families referred because of unexplained familial thrombophilia, APC resistance may be found in 40% to 60% of subjects. (6)

Individuals with A PC resistance have a seven fold increased risk of venous thrombosis. Because of the high allele frequency of the mutated factor V gene, homozygous carriers will not be extremely rare as in other types of hereditary thrombophilia. It is unknown whether the homozygous state confers a higher risk than the heterozygous state. We have estimated the risk of thrombosis and the clinical features of patients who were homozygous for factor V Leiden. These patients were identified in a large, population-based, case-control study on deep-venous thrombosis. (6)

Dahlback discovered a novel mechanism for familial thrombosis in 1993. It is characterized by an inherited resistance to the anticoagulant action of activated protein C (APC).2,S Studies report that the discovery of activated protein C resistance (APC-R) has raised the yield of diagnosed coagulation abnormalities up

to 64% in thrombosis patients and up to 8% in the general population. The wide range is postulated because of differences in selection criteria, ethnic background of the populations studied, and laboratory methods used. APC-R is by far the most common inherited thrombophilia. [1.3·5.7-9].

Pregnancy increases the risk of thrombosis. APCR phenotype has been associated with venous thromboembolism (VTE), the primary cause of maternal death in developed countries. In normal conditions, APC inactivates the coagulant protein active FV(a) by cleaving in an ordered sequence specific sites of FV(a). The first cleavage site is Arginine (Arg) 506, and the second is (Arg) 306 followed by (Arg) 679. Mutations in the FV gene have been related to APCR. FVL is reported in about 90% of patients with APCR in the general population. Other SNPs in the factor V gene which may contribute to inherited APCR either independently or found in association with the FVL mutation include Cambridge Arg306, Hong Kong, Arg306, the Arg679, and the haplotype (H) R2 and R3 polymorphisms. However, reports on the contribution of these mutations to the APCR phenotype are conflicting. The pathophysiology underlying APCR not caused by the FVL mutation is still not completely understood. In different studies, it has been suggested that acquired factors might be the cause of APCR in the absence of FV Leiden. A number of coagulation factor scan affect the activated partial thromboplastin time (aPTT). Previous literature suggested a possible positive correlation between levels of factors V, VIII and IX and acquired APCR. Protein S and protein C, levels can (or may) affect acquired APCR, but their influence on the resistance seems to be still within the range of normal levels ([3])

A variety of inhibitory system, which inactivates either serine proteases or cofactors, inhibits the coagulation process. A dynamic inhibitory system is generated when thrombin binds to its co-factor thrombomodulin, which is constitutively present on the vasculature, and activates protein C to a serine

protease activated protein C.[9] Activated protein C inhibits the coagulation reaction by the proteolytic cleavages and concomitant inactivation of factor V, factor Va, factor VIII, and factor VIIIa.[4,5]

Normal pregnancy is associated with significant alterations in all aspects of the Virchow triad: Venous stasis, endothelial damage, and enhanced coagulation, thereby shifting the equilibrium towards the pro-thrombotic state.[10] These include increasing concentrations of most clotting factors, decreasing concentrations of some of the natural anticoagulants, and reducing fibrinolytic activity. Indeed, there is a significant decrease in protein S activity and progressive increase in resistance to activated protein C in second and third trimesters. [11] Consequently, the overall balance of hemostasis tilts towards apparent hyper coagulability as pregnancy progresses even up to puerperium. [12]

Hypercoagulability in pregnancy is critically, essential, for the provision of adequate hemostasis to the placental site and certainly acts in synergy with uterine contractions to prevent post-postpartum hemorrhage and avert maternal death. [12]

2.2 Complications induced by APCR in pregnancy

Normal pregnancy is characterized by acquired activated Protein C resistance, but this hypercoagulable state doesn't induce thrombotic complications and doesn't need antithrombotic prophylaxis. Instead, the inherited activated Protein C resistance induced by FV Leiden has often complicated pregnancy. The mutation induce a three to four fold higher risk of an adverse pregnancy outcome [13] and has a stronger association with severe and early onset preeclampsia. [14, 15] Also, recurrent miscarriages, defined as three early consecutive losses or two late pregnancy losses after 12weeks gestational age [16] has been shown to be associated with APCR. The data on the risk of intrauterine fetal growth restriction (IUGR) are more limited and conflicting [17, 18]

Placental abruption or maternal venous thrombosis during pregnancy or postpartum is another described complications [19, 20]. The causes of these are unknown but all

of them may be associated with abnormal placental vasculature and disturbances of hemostasis leading to inadequate maternal-fetal circulation [21]. Another possible mechanism is cell death and inhibition of trophoblast cells growth induced by activated coagulation factors [22]. Clinical factors that increase these complications risk in pregnancy are: maternal age, maternal weight, high parity, major current illness and operative delivery

2.3. Haemostatic changes in pregnancy

Normal pregnancy and puerperium are characterized by a marked increased in the procoagulant activity in maternal blood [23]. Virchow's triad in normal pregnancy is characterized by venous stasis, venous hypotonia or vascular damage and hypercoagulability. The most blood coagulation factors and fibrinogen increase during pregnancy: FVII and FX are mild increasing, Fibrinogen and FVIII are 2 fold increasing, von Willebrand Factor increases 3-fold and remains elevated some period post partum and FV gradual rises [24].

FXII, X and IX increase progressively in contrast with FXI that is the only blood coagulation factor that decrease. Tissue factor (TF) no change and it has a VTE protecting role in pregnancy. Another hypercoagulability causes in pregnancy are the natural coagulation inhibitors changes: total and free protein S decrease about 30% and may remain decreased for at least up to 2 months postpartum, protein C remain constant or increase but Heparin cofactor II and Thrombomodulin increase in pregnancy. Level of ATIII remains stable during pregnancy. Fibrinolytic capacity is diminished during pregnancy, mainly because of markedly increased levels of plasminogen activator inhibitor-1 (PAI-1) from endothelial cells and plasminogen activator inhibitor-2 (PAI2) from the placenta. [25]

The changes in the haemostatic system progress with pregnancy evolution and are maximal around term; its help in maintaining placental function during pregnancy, minimizing intrapartum blood loss and preparing the haemostatic challenge of delivery. Haemostatic system returns to non pregnant state in (4 – 6 weeks) post-

delivery. The incidence of VTE in pregnancy is 1/1000 deliveries (6 fold higher than in general female population of child-bearing age). An important anticoagulant mechanism changed is acquired APC resistance that was reported in up to 50% of normal pregnancies. The cause of this change is the increase level of FVIII and FV, decrease level of PS or APC inhibitors. [26].

2.4. Activated Protein C Resistance and factor V leidin

The Protein C/Protein S Anticoagulation Pathway The pathway to inhibit fibrin formation by degrading selected coagulation factors is as complex as the cascade leading to the conversion of fibrinogen to fibrin . Thrombin (factor IIa), the same factor that converts fibrinogen to fibrin and activates platelets, is also responsible for initiating the pathway to inhibit fibrin formation. Thrombin binds to thrombomodulin on the blood vessel wall. When thrombin is bound to thrombomodulin on the surface of an endothelial cell, it becomes activated and can convert protein C to its activated form (activated protein C). [27]

Activated protein C is only effective when it is bound to its cofactor, protein S. Protein S is available as a cofactor for protein C only when it is not bound to C4b binding protein. In the basal state, approximately 40% of protein S is free (unbound) and thereby available to serve as a cofactor for activated protein C. The activated protein C/ protein S complex degrades factors Va and VIIIa, and their loss is associated with a decrease in fibrin formation and, therefore, a reduction in the ability to form a fibrin clot. Although not shown in Figure 1, factor V itself acts as a cofactor for activated protein C/protein S in the degradation of factor VIIIa.2 In addition, a receptor on the endothelial cell, called the endothelial cell protein C receptor, enhances protein C activation by the thrombin-thrombomodulin comple.[27]

2.5. Activated Protein C Resistance

Activated protein C normally degrades activated factors Va and VIIIa by proteolytic cleavage at specific arginine residues. Individuals with activated protein C resistance have a mutated factor V, such that it is resistant to degradation by activated protein C. More than 95% of cases are due to a point mutation, known as the factor V Leiden mutation, at 1 of the 3 arginine cleavage sites in the factor V gene. Two additional, very rare factor V mutations at other arginine cleavage sites have been identified recently, factor V Hong Kong8 and factor V Cambridge. Factor V Cambridge can cause activated protein C resistance, but factor V Hong Kong has not caused activated protein C resistance in the few cases reported to date. Other factor V mutations are also under investigation. Mutations in the factor VIII gene causing resistance to activated protein C are theoretically possible but have not yet been described. Very rarely, activated protein C resistance with thrombosis has developed as the result of an autoantibody (inhibitor) against activated protein C, without an underlying genetic mutation in factor V. [28]

2.5.1. The Clinical Significance of Activated Protein C Resistance

The factor V Leiden mutation is present in 3% to 5% of the general white population in heterozygous form.13–17 It is less common or rare in other races and ethnic groups, especially those of African or Asian ancestry. The presence of the factor V Leiden mutation confers a genetic risk for thrombosis, which is primarily venous. The risk for venous thrombosis is approximately 3- to 10-fold in individuals who are heterozygous for the factor V Leiden mutation. Because the mutation is present in as many as 1 of 20 in the white population, homozygotes are not rare. Homozygous individuals have been reported in different studies to have an approximately 80-fold risk over baseline for thrombosis. [29]

In the population of all patients with a venous thrombosis, which includes deep vein thrombosis and pulmonary embolism, approximately 20% of cases are positive for the factor V Leiden mutation. Amongthe population of individuals who have a

family history of thrombophilia, approximately 50% have the factor V Leiden mutation.6,7,20 Thus, this particular mutation accounts for a significant percentage of people with a thrombotic event or a family history of thrombosis. Individuals who are positive for the factor V Leiden mutation in heterozygous or homozygous form oftenneed the presence of a second risk factor, which can be genetic or acquired, to produce a thrombotic event.[30]

Many of the acquired causes are well known, and include malignancy, trauma, surgery, the use of oral contraceptives or estrogen replacement therapy, and the presence of an antiphospholipid antibody, either as a lupus anticoagulant, an anticardiolipin antibody, or both. Patients who are double heterozygotes for the factor V Leiden and prothrombin G20210A, another high-incidence mutation in the white population, have a further increased risk for thrombosis.[31]

The presence of elevated homocysteine, occurring as a result of a genetic cause or an acquired cause (such as low levels of vitamins B6,B 12, or folate), can further increase the risk of thrombosis with the factor V Leiden mutation. The thrombosis risk with the factor V Leiden mutation also increases with age. The increased risk associated with oral contraceptive use combined with the factor V Leiden mutation is synergistic rather than additive. [32]

The risk of arterial thrombosis with the factor V Leiden mutation is uncertain, but it appears that factor V Leiden may be more prevalent among myocardial infarction patients who do not have atherosclerosis and/or young patients with certain other risk factors (smoking, hypertension, obesity, high cholesterol, or diabetes) when compared with control groups.[33–35] The incidence and severity of thrombosis with heparin-induced thrombocytopenia did not appear to be affected by the presence or absence of factor V Leiden. [36] A few studies suggest that among individuals with factor V Leiden, those with type O blood may have less risk for thrombosis than individuals with type A, B, or AB blood.[37,38] One study found that although factor V Leiden was associated with an increased incidence of deep vein thrombosis

overall, the incidence of deep vein thrombosis was actually significantly decreased.[39] The thrombi with factor V Leiden were predominantly distal to the iliofemoral veins. Since pulmonary emboli arise more often from thrombi in the ilio-femoral veins than from more distal thrombi, this may help explain why the prevalence of factor V Leiden/activated protein C resistance is lower among patients with isolated pulmonary embolism (8.9%) than in patients with isolated deep vein thrombosis (18.8%).[40]

2.5.2. APC Resistance, a Common Risk Factor for Thrombosis

Activated protein C (APC) is a key anticoagulant enzyme needed for the proper down-regulation of blood coagulation. A poor anticoagulant response to APC, denoted APC resistance, is a recently described blood defect found to be a major risk factor for venous thromboembolism in Western societies. At least 90% of cases with the APC resistance phenotype can be explained by a point mutation in the gene for coagulation factor V. The mutation predicts the synthesis of an abnormal factor V molecule (termed FV:Q506 or FV Leiden) that is partially resistant to inactivation by APC, causing a life-long disposition to a hypercoagulable state. APC resistance due to the presence of the FV:Q506 allele is inherited as an autosomal dominant trait and has a prevalence of 2-13% in the general population. Frequencies of APC resistance among patients with venous thrombosis, depending on the selection criteria, range from 20-60%. The high prevalence of APC resistance and the availability of simple blood tests to detect this disorder, raises the question whether more general screening for APC resistance should be performed in conjunction with surgery, pregnancy, use of oral contraceptives and other established risk factors for thrombosis. [41]

Venous thromboembolism; the formation of an obstructive mass of clotted blood in the venous part of the circulatory system is known as venous thrombosis. The mass itself is called a thrombus and is composed of platelets, blood cells and fibrin. A thrombus which breaks loose and is carried away with the bloodstream is called an

embolus. When caught in the blood vessels of the lung it may develop into pulmonary embolism, the most feared complication of venous thrombosis. Venous thromboembolism is a major health problem in Western societies, constituting the third most common cardiovascular disease after acute ischemic heart disease and stroke. [41] The incidence has increased steadily in recent centuries, perhaps due to longer life-spans and the adoption of more sedentary habits. In the USA, venous thromboembolism accounts for more than 250,000 hospitalizations a year, corresponding to an incidence of about one per 1,000 individuals. The annual death rate due to pulmonary embolism is estimated to be 50,000.[41]

Thrombogenic risk factors As described by Rudolf Virchow more than a century ago, there are three primary pathogenic risk factors for venous thrombosis: a reduced blood flow, vessel wall damage, and a change in blood components .

Any one of these risk factors potentiates the other and creates a hypercoagulable state in which the balance between procoagulant and anticoagulant forces has shifted in favor of coagulation. [42,43]Hypercoagulability and venous thrombosis tend to develop in conjunction with circumstantial or acquired risk factors such as surgery, pregnancy, use of oral contraceptives, immobilization, cancer and old age. It is also known that genetic risk factors often play an important role in the pathogenesis, since as many as 20-40% of patients referred to a specialist laboratory may have a family history of thrombosis. [44-46]

However, genetic defects associated with an inherited tendency to develop thrombosis (thrombophilia) were, until recently, identified in only a few percent of all thrombosis patients.[47]

Novel defect in the protein C anticoagulant pathway The diagnostic situation for inherited thrombophilia improved dramatically in 1993 with the discovery of a novel defect in the protein C anticoagulant pathway. Based on the hypothesis that a poor anticoagulant response to activated protein C (APC) might predispose to thrombosis, a Swedish research group led by Björn Dahlbäck measured the

anticoagulant activity of exogenously added APC in an APTT-based assay.[48] In a normal response, the addition of APC to plasma induces a prolonged clotting time. This occurs because APC cleaves and inactivates two critical coagulation proteins, factors Va and VIIIa. However, when the assay was run on plasma from a middle-aged man suffering from recurrent episodes of venous thrombosis, the result showed a much shorter prolongation of the clotting time than expected.[48]

Several of the man's relatives demonstrated a similar poor anticoagulant response to APC and family studies suggested that this disorder, denoted APC resistance, was inherited as an autosomal dominant trait.[48,51] Subsequent investigations carried out in Western countries showed that APC resistance was present in 20-60% of all cases of venous thromboembolism and that it was highly prevalent in the general population (1-7%).[49-52] These results proved APC resistance to be the most prevalent cause of thrombophilia, being larger than the sum of all other previously established genetic risk factors, including antithrombin, protein C and protein S deficiency[49-52].

Mutation in the factor V gene explains APC resistance The search for the molecular mechanism of APC resistance led to the isolation of a protein from normal plasma, which was able to correct APC resistance in a dose-dependent manner. This protein was identified as factor V, suggesting that APC resistance was caused by a genetic defect in the factor V gene.37 Other studies reached the same conclusion and a point mutation that predicts the replacement of arginine (R) at position 506 in the factor V molecule with glutamine (Q) was soon identified.[53-55] The mutated protein, denoted FV:Q506 (or FV Leiden), is activated in a normal way and retains normal procoagulant activity, although it is partially resistant to APC cleavage and inactivation resulting in a disposition to a hypercoagulable state. At least 90% of APC resistant cases are explained by this mutation.[56]

Diagnostic breakthrough in thrombophilia The discovery of APC resistance and the identification of the FV:Q506 mutation as its main cause means, that a genetic

explanation can now be identified almost as often as non-genetic risk factors in thrombosis patients This diagnostic breakthrough, in combination with the availability of simple laboratory tests, offers a powerful tool for preventing venous thromboembolism. This monograph reviews the APC resistance phenomenon and describes the major tests for its phenotype and the FV:Q506 genotype. The use of these tests in the clinical environment will help establish guidelines for therapy and prophylaxis, which hopefully will lead to reduced morbidity and mortality in thrombophilic patients.[56]

2.6. Natural substrates of APC, factors VIIIa and Va

Factors V and VIII are two large, relatively unstable, plasma proteins of about 330 kDa, with similar structure and function.[57-59] Factor V is an essential component for the rapid conversion of prothrombin to thrombin, whereas factor VIII is needed to accelerate the activation of factor X to factor Xa. The essential role of these non-enzymatic cofactor proteins in hemostasis is evidenced by the severe bleeding tendency associated with their deficiency.[57,60] Both factor V and factor VIII are synthesized mainly in the liver and circulate in plasma as inactive molecules with little or no procoagulant activity. A unique feature of factor VIII is that it circulates in a stabilizing, noncovalent complex with the von Willebrand factor, an adhesive protein that is important for the proper function of platelets.[57] The plasma concentration of factor V is about 10 μg/ml, which is up to a 100-fold higher than that of factor VIII (0.1-0.2 μg/ml).[61] About 20% of the total amount of factor V in blood is synthesized by megacaryocytes and stored in platelets. This stored form of factor V is released in conjunction with platelet activation and has an important role in normal hemostasis. The genes for factor V and VIII are located on chromosomes one and X respectively. They code for mature, single-chain proteins of roughly 2200 amino acids. [57,58]

Prior to secretion into the bloodstream, the factor VIII molecule is processed to a calcium ion-linked heterodimer, whereas factor V circulates as a single-chain

protein. [57,58] Computer-aided comparison of the primary amino acid sequence of factors V and VIII reveals a high degree of homology, with an overall identity of about 30%.24 Both proteins contain several types of similar internal repeats, termed A1-A2-B-A3-C1-C2 .[57,58]

2.6.1. Activation of factors V and VIII

Factors V and VIII are activated through limited proteolysis by thrombin or factor Xa.[57,58]During its activation, factor VIII is released from the protective influence of the von Willebrand factor and converted to a calcium ion-dependent trimer (A1, A2 and A3-C1-C2). The active factor V molecule (factor Va) is a dimer that consists of a heavy chain (A1-A2), non-covalently linked via calcium ions to a light chain (A3-C1-C2) (Figure 5). The activated factors VIIIa and Va bind to negatively-charged phospholipid in the presence of calcium and serve as cofactors/receptors for factors IXa and Xa respectively. The importance of these multimolecular complex assemblies, better known as the tenase and prothrombinase complexes, is evidenced by the over 100,000-fold increase in the combined rate of activation of factor X and prothrombin when compared to the activation catalyzed by their respective enzyme alone.[59]

2.6.2. Inactivation of factors Va and VIIIa APC

Effectively degrades phospholipid-bound factors Va and VIIIa. In contrast, the native forms of the proteins are poor substrates for APC. The inactivation of factor Va takes place through the APC-mediated cleavage in the heavy chain of the molecule of three peptide bonds at Arg506, Arg306 and Arg679 (Figure 6).[62]

Cleavage at Arg506 is needed for the efficient exposure of the cleavage sites at Arg306 and Arg679. The lipid-dependent cleavage at Arg306 appears to be the major inactivating cleavage site and results in a loss of about 80% cofactor activity, whereas cleavage at Arg679 is lipid-independent and is responsible for the loss of most of the remaining cofactor activity.34 Potential structural differences between platelet factor Va and plasma factor Va may influence the extent to which the

cofactor is cleaved initially at Arg306.35 APC inactivates factor VIIIa by cleavages at Arg336, Arg562 and Arg734. The main loss of factor VIIIa cofactor activity is associated with the cleavage at Arg562.36.

2.7. Molecular explanation of APC resistance

The initial observation that normal factor V mixed with APC resistant plasma was able to correct the APC response in a dose-dependent manner, suggested to several independent research groups that APC resistance was due to a defect in the factor V molecule.[53,67,68] However, the precise molecular explanation was discovered first by a Dutch group led by R. Bertina.[53] The APC resistance phenotype in this seminal study was linked to a single-point mutation in the factor V gene, which substitutes G (codon CGA) with A (codon CAA) at nucleotide 1691 in exon 10.[53] This mutation replaces Arg (R) with Gln (Q) at position 506 in the factor V molecule, thus modifying one of the three APC cleavage sites (Figure 6). The mutant FV:Q506 molecule expresses normal procoagulant activity when activated by thrombin or factor Xa, although its rate of inactivation is about 10-fold slower than that of normal factor Va.[63-66]

This "resistance" to degradation by APC allows for a larger duration of thrombin generation, which is reflected by increased levels of prothrombin fragment 1+2, thrombin-antithrombin (TAT) complex and D-dimer.[43-46] Recent data also suggest that a reduced ability to slow down thrombin generation may stabilize a blood clot by weakening the profibrinolytic effect of APC.48 An antifibrinolytic mechanism could thus be an additional factor contributing to the prothrombotic tendency observed in APC resistant patients. The fact that mutant FVa:Q506 can still be inactivated by APC cleavage at Arg306 and Arg679 might account for the relatively mild hypercoagulable state observed in APC-resistant individuals and help explain why additional genetic and/or acquired risk factors are required for thrombosis to develop.[65]

2.8. Hypercoagulable state and thrombophilia

A hypercoagulable state is a condition that favors coagulation, as recognized by increased thrombin generation. Hypercoagulability can be due to a number of factors, which can be either inherited (primary) or acquired (secondary) (Table 2). Thrombophilia is the clinical term for a hypercoagulable state that causes an increased tendency to thrombosis. Several genes have been implicated with inherited thrombophilia, although only factor V (APC resistance), antithrombin, protein C and protein S have been clearly linked to an increased risk of venous thromboembolism (Table 3)[.47,69] Of these, APC resistance is the most common, both among patients and in the general population (Table 4). [49-52]

Table (2.1,2)

Primary and Secondary hypercoagulable states:

Secondary hypercoagulable states Table 2	Primary hypercoagulable states Inherited thrombophilia (Table 3
Advanced age	Established: APC resistance (factor V:Q506)
Heart disease	Antithrombin deficiency
Immobility	Protein C deficiency
Lupus anticoagulants	Protein S deficiency
Malignancy	Non-established
Obesity	Dysfibrinogenemia
Oral contraceptives	Plasminogen deficiency
Pregnancy	Elevated PAI-1 Heparin Cofactor II deficiency
Trauma and surgery	Factor XII deficiency
Varicose veins	Hyperhomocysteinemia

2.9. Diagnosing APC resistance

The development of a simple, APTT-based assay that measures the anticoagulant response in plasma to added purified APC, facilitated the characterization of the

APC resistance phenotype.8 In the classic test kit, two APTT reactions are performed, one in the presence of a carefully-defined quantity of APC and the other in its absence.[70]

The relationship between the two clotting times is expressed as a ratio, called the APC ratio. Healthy individuals have an APC ratio in the range 2-5, whereas APC-resistant individuals are recognized by an APC ratio below or equal to about 2. The precise cut-off for a diagnosis may vary slightly depending on the instrument type used as well as the individual condition of the instrument.[71-74] The phenotypic APC ratio reflects the severity of the hypercoagulable state and provides information on the thrombotic risk associated with inherited and possibly acquired APC resistance. A modified APC resistance test (IL Test™ APC™ Resistance V), which exclusively detects factor V-related APC resistance is available, i.e. APC resistance due to the FV:Q506 mutation.[75]

The assay modification involves a predilution of plasma samples with an excess of stabilized factor V-deficient plasma (Factor V Reagent Plasma) containing a heparin antagonist. Since the predilution with Factor V Reagent Plasma normalizes the basal APTT reaction, it safely allows for APC resistance-testing of plasma from patients on oral anticoagulant or heparin therapy. It also produces a complete discrimination for FV:Q506, which makes the modified assay highly suitable for factor V mutation screening. Test results are expressed as an APC-V ratio calculated in the same way as the APC ratio obtained from the classic test. The APC-V ratio provides genotypic information concerning factor V and is generally lower than the APC ratio for the same sample, regardless of the instrument used. Typical APC-V ratio ranges for different factor V genotypes are 2.2 - 3.2 for normal FV:R506, 1.4-1.8 for heterozygous FV:Q506, and 1.1 - 1.3 for homozygous FV:Q506.[75].

2.10. The origin of the FV:Q506 mutation

Several investigators have suggested that the high prevalence of the FV:Q506 mutation could be due to the evolutionary advantage it would confer, which has helped to maintain and spread the mutation. [77-79] It is possible that the selective disadvantage of a life-long hypercoagulable state could be balanced by, for example, the protection against excessive blood loss during delivery and menstruation. The selective risk of the FV:Q506 mutation would also be of less historical importance, as people in ancient times were not exposed to modern risk factors for thrombosis (e.g. oral contraceptives, surgery, sedentary life-style etc.). The high allelic frequency of FV:Q506 in Caucasian populations and its linkage to different polymorphisms in the factor V gene, supports the hypothesis that the mutation occurred as a single event in the ancient European population.[53,80,81,82] The time of this event would be approximately 30,000 years ago, i.e. after the diversion of Africans from non-Africans (140,000 years ago) and after the diversion of Caucasoid from Mongolic populations (70,000 years ago), but before the diversion of Caucasian subpopulations. However, the possibility of recurrent mutations in other races is not altogether unlikely, since the FV:Q506 mutation involves a CpG dinuclotide, which is an established hot-spot for mutation.[83]

2.10.1 .When to test for APC resistance phenotype and FV:Q506 genotype

Up to now, the classic APC resistance test has been used mainly as a simple screening assay for the FV:Q506 mutation. However, several limitations have been reported for this application. Firstly, this test has a sensitivity and specificity for the mutation which is usually in the range 75-90%.[84,85]Secondly, the test is only reliable if the basal APTT-reaction is within the normal range,[86] which therefore disqualifies many APC-resistant patients on anticoagulant therapy from testing.[87] As the classic APC resistance test stands today, it is not recommended for FV:Q506 mutation screening if the modified test can be performed instead (see below). The question that arises is when should the classic test be used?

Interestingly, the APC ratio is not a one-variable reflection of the APC response in vivo, but rather an indication of an anticoagulant system response that in general seems to decrease under a hypercoagulable state.[88] A poor anticoagulant response to APC, independent of the FV:Q506 mutation, may therefore be a thrombotic risk factor or risk marker in a wide range of conditions, including venous thromboembolism,[56,88] antiphospholipid protein syndrome,209 second trimester miscarriage,[90] systemic sclerosis,[89] ischemic stroke,[91,92]occlusion after vascular surgery,[93] pregnancy[94] and the use of oral contraceptives.[95-97] Future studies will clarify whether the phenotypic APC ratio obtained from the classic test may serve as a predictor of venous and arterial thrombotic events.[95-97]

2.11.1 Management of APC resistant patients

A potentially large number of thrombosis-prone individuals with APC resistance will most likely be identified in the near future. This of course raises the question of patient management. At present there are no established guidelines for managing thrombotic patients with APC resistance, although it is generally agreed that they should be treated in the same way as the patients with antithrombin, protein S and protein C deficiency.[98] An acute thrombotic episode should be treated conventionally with heparin for 5 to 10 days, followed by an oral anticoagulant (warfarin) within 24 hours to produce an International Normalized Ratio (INR) of 2.0 to 3.0. Patients should be given general advice on how to minimize the thrombotic risk, including dietary advice, cessation of smoking and avoiding long periods of immobility. Thrombophilic women should avoid oral contraceptives and all patients should be notified that they may require special treatment prior to surgical, medical or obstetric procedures that carry an increased thrombotic risk. Women with a history of thrombosis and who have a known genetic defect may require anticoagulation throughout pregnancy, preferably by using dose-adjusted subcutaneous heparin.[99] As a rule, all thrombophilic women should be offered thromboprophylaxis in conjunction with delivery and puerperium. Women with no

previous history of thrombosis, but who do have a genetic defect, require individual consideration. Homozygous and heterozygous patients with a second anticoagulant defect should be given preventive therapy in all risk situations and long-term therapy should be considered if thrombosis is recurrent. When a woman has experienced venous thrombosis after oral contraceptive use it is recommended that she is tested for the possible presence of the FV:Q506 mutation. If she is heterozygous for the mutation she should be carefully informed about her thrombotic risk and counseled about the type of contraceptive she should use in the future. The mere fact that she has had a thrombosis indicates that the risk in her case is significant. If she is homozygous for the mutation, she should be strongly recommended to discontinue the use of oral contraceptives. As in any investigation of a young patient with venous thrombosis and APC resistance, it is also recommended to search for other causes of inherited thrombophilia. Because only a proportion of the subjects with a heterozygous defect develop thrombosis, it is unjustifiable to put symptom-free family members on antithrombotic prophylaxis solely on the basis of having a genetic defect. It is, however, essential that these asymptomatic family members are carefully counseled with respect to their defect and offered short-term prophylaxis in special situations where there is an extra risk of thrombosis.[99].

2.12.1. Factor V Leiden mutation and its impact on pregnancy complications

During pregnancy the gentle haemostatic balance shifts towards enhanced coagulation, resulting in an increased risk of venous thromboembolism. Pulmonary embolism is the main cause of maternal mortality in developed countries [100]. As a result of influencing placental perfusion, thrombotic processes have been described as an important pathogenetic factor in some severe obstetric conditions (such as preeclampsia, intrauterine growth restriction (IUGR) and placental abruption). Thrombotic events have also been found in fetal circulation. Both congenital and acquired thrombophilias are implicated in pathophysiological processes associated

with thrombotic damage of the placenta as well as with an increased risk of venous thromboembolism (VTE) (Tab. 1). Activated factor V (FVa) acts as a cofactor to activated factor X (FXa) in the conversion of prothrombin. The procoagulant function of FVa is down-regulated by the serine protease activated protein C (APC) for haemostatic maintenance. The activation of protein C begins on the surface of endothelial cells through the thrombin-thrombomodulin complex. [101]

Factor V Leiden mutation is most common thrombophilia and its prevalence of heterozygosity in Caucasian. [101]

2.12.2. Previos study

prospective study enrolled between October 2006 and September 2009.

The aim of this prospective study was to find the association between the factor V Leiden mutation and adverse pregnancy [102]

study included 52 women with a singleton pregnancy and a history of one unexplained abortion in the first trimester before thrombophilia screening

The heterozygous factor V Leiden mutation was confirmed in 11 women

Negative results were obtained in 41 women. [102]

These 11 women with heterozygous factor V Leiden was 29 years (range 19–34 years)

Study shown:

- IUGR 3women (27.3%).
- Preeclampsia1 woman (9.1%).
- Eclampsia 0 0 Birth before 37th week 1 woman (9.1%).
- Placental abruption 1 (9.1%).
- Vaginal delivery3 women (27.3%).

Chapter three

Materials and Methods

3. Material and methods

3.1. Study design

Descriptive analytical case control study conducted in Shendi city to evaluate role of APCR in women with complicated pregnancy

3.2. Study area

This study conducted at Shendi city, in northern state, it is about (45Km) southwest of the ancient city of Meroe. Located in the River Nile state, Shendi is center of Jaaliin tribe. any boo an important historical trading center. on the west bank Al-matamma. major traditional trade route across the bayuda desert connected Al-matamma to Marawi and Napata (250Km) to the Northwest.

3.3. Study population

34 Ladies with placental complicated pregnancy

3.4. Inclusion criteria

Women with complicated pregnancy

3.5. Exclusion criteria

Women under warfarin thereby

3.6. Data collection tools

Questionnaire contain closed questions

3.7. Sample processing

Allowed to Patient take rest for 10 min prior sampling, collected venous blood in sodium citrate(volume ratio 9+1).mixed gently, avoided foam formation, then centrifuged immediately at no less than 2000x g at least 20 min at room temperature. excluded heamolytic plasma sample, samples freezed rapidly at -80ºC.[104]

3.8 Resistance to activated protein C [APCr Assays]

Acticlot Protein C Resistance is a phenotypic assay with the sensitivity to distinguish between homozygous and heterozygous carriers of Factor V Leiden, without using molecular (PCR) methods. [103]

3.9. Principle of the method

The acticlot protein C resistance assay is a plasma-based functional clotting assay and differs from other functional (APC) resistance tests by acting specifically on the prothrombinase complex level. It is based on a FV-dependent prothrombin activator isolated from snake venom. Robustness and specificity of the assay is enhanced by elimination of possible disturbing influences by factors upstream the coagulation cascade and independency from calcium. Interference from (UFH), (LMWH) and pentasaccharide in the blood sample is precluded by a heparin inhibitor added to reagents 1 and 2. Sample plasma is pre-diluted with reagent 4 (dilution plasma) and incubated at 37 °C with (FV) activator from snake venom (RVV-V from Daboia russelli). Coagulation is triggered by the addition of a (FV) dependent prothrombin activator from snake venom from Notechis scutatus in the absence of calcium. The clotting times are recorded and the ratios (clotting time in the presence of APC/clotting time in the absence of (APC) are calculated.[103]

3.10. REAGENTS[105]

Reagent.1

APC / RVV R1 -V (+APC) Reagent**,** (APC, RVV-V, Polybrene, Hepes, BSA) 3 vials (lyophilisate, to be reconstituted in 2.0 ml of deionized water per vial)

Reagent.2

R2 RVV-V (-APC) Reagent(RVV-V, Polybrene, Hepes, BSA)

3 vials (lyophilisate, to be reconstituted in 2.0 ml of deionized water per vial)

Reagent.3

R3 PTA Reagent (Prothrombin Activator, EDTA, Hepes, BSA)

3 vials (lyophilisate, to be reconstituted in 4.0 ml of deionized water per vial)

Reagent.4

(R4 Dilution Plasma (Processed Human Plasma)

3 vials (lyophilisate, to be reconstituted in 2.0 ml of deionized water per vial)

Incubate reconstituted solutions R1-R4 in closed vials for 30’ at room temperature

and swirl gently before use.

3.11 Procedure

Thawed samples were mixed for homogenization. The clotting time was determined in the presence of Activated Protein C and also in the absence of Activated Protein C and the ratio was calculated according to the following scheme:[105]

		+ APC	-APC
	Sample or control plasma	**30 µL**	**30 µL**
R4	**Dilution Plasma**	**20 µL**	**20 µL**
		mix prior to use	**mix prior to use**
R1	**APC/RVV-V (+APC) Reagent**	**50 µL**	**-**
R2	**RVV-V (-APC) Reagent**	**-**	**50 µL**
	Incubation	**3 min, 37°C**	**3 min, 37°C**
R3	**PTA Reagent**	**50 µL**	**50 µL**
		Determine clotting time	**Determine clotting time**
	Ratio calculation =	**Clotting time + APC/Clotting time – APC**	

3.12.1 Clotting time for samples and controls in the presence of APC:

Determination of a clot in the in the presence of APC, 30 µL of plasma or control was add to 20 µL of the diluent, these reagents were mixed well, 50 µL of RVV-V (+APC) was added to the above mixture, the reactant was incubated for 3 min at 37°C, 50 µL of clot initiating reagent (PTA) was added and the clotting time was determined. [105]

3.12.2 Clotting time for samples and controls in the absence of APC

Determination of a clot in the in the presence of APC, 30 µL of plasma was add to 20 µL of the diluent, these reagents were mixed well, 50 µL of RVV-V (-APC) was added to the above mixture, the reactant was incubated for 3 min at 37°C, 50 µL of clot initiating reagent (PTA) was added and the clotting time was determined. [105]

3.13. APC-R calculation:

APC-R was calculated as a ratio between clotting time with APC/Clotting time without APC[105]

3.14. Reference range:

0.9 - 1.2 homozygous

1.2 - 2.3 heterozygous

>2.9 negative

3.15. Results interpretation

All quality control procedure were adopted to get the validation of the assay, each test was done parallel with negative and positive controls; the acticlot Protein C Resistance Control Plasmas (REF 840C) as a control reference was done, the negative control. [105]

3.16. Ethical consideration

This informed concent of the seected individual to the was taken after being informed with all detailed objectives of the study and it is halth emphasis in future

3.17. Data analysis

The data collected code in master sheet and procesed using SPSS programme (Mean, standard deviation and P.value by using independent T test).

3.18. Data interpretation

The result of this study shown in tables.

Chapter four

Results

Results

This study was conducted to evaluate factor V Leiden by using APC-R test on 43 women with complicated pregnancy, fount that 73% (mean 3.96) of the under study group were negative for factor V Leiden mutation, 27% (mean 2.1) heterozygous(carriers) and no homozygous as shown in table no (4-1)

Table (4-1) Mean of APC-R in women with complicated pregnancy

	Std. Deviation	Mean	Percent	Frequency
Negative	25	73%	3.96	0.963
Heterozygous	09	27%	2.1	0.468
Total	34	100%	3.4	1.194

The results revealed 88% were miscarriage and 12% were pre term labour according to table no 4-2

Table (4-2) Types of complication Frequency in carriers group

	Frequency	Percent
Miscarriage	8	88%
PTL	01	12%
Total	09	100%

The type of complicated pregnancy that we found among 34 women with complicated pregnancy were miscarriage, pre term labour(PTL), preeclamsia and stillbirth as 73%, 09%, 09% and 09% respectively as shown in table no 4-3

Table (4-3) The type of complicated pregnancy Frequency

	Frequency	Percent
Miscarriage	25	73%
PTL	3	9%
Preeclamsia	3	9%
Stillbirth	3	9%
Total	34	100%

The age at complicated pregnancy that founded among 34 women, 8 women (23%) between 18-28.and same account between 28-38.

16 women (54%) between 38-48.

Mean of age among complicated pregnancy (29)

Table (4-4) Women with complicated pregnancy age Frequency

Age	Frequency	Percent
18 – 28	8	23%
28 – 38	8	23%
38 – 48	16	54%

Mean of carrier age (30) beween (23-35year).age mean of wild type (29) between (19-48).

Table (4-5) Mean of age among cariers and wild

	Cariers	Wild ytpe
Mean	30	29

Chapter five

Discussion

Conclusion

Recommendations

5.1 Discussion

This study shew 73% (mean 3.96) of the under study group were negative for factor V Leidine mutation, 27% (mean 2.1) heterozygous (carriers) and no homozygous. Study revealed revealed 88% of carriers were miscarriage and 12% were pre term labour

This study agree with prospective study enrolled by Ľubica Hammerová1, Ján Chabada1and Juraj Drobný between October 2006 and September 2009 ,to find the association between the factor V Leiden mutation and adverse pregnancy [(102)] at 52 women with a singleton pregnancy and a history of one unexplained abortion in both studies no existence of homozygous

Also Ľubica Hammerová1, Ján Chabada1and Juraj shown the heterozygous factor V Leiden mutation was confirmed in 11 (21%) women Negative results were obtained in 41(79%) women. [(102)]

our study shown (27%) heterozygous and (73%) wild type

Ľubica Hammerová1, Ján Chabada1and Juraj shown

IUGR 3women (27.3%) higher than this study that shown (9%)

The above mentioned study shown Preeclampsia1 woman (9.1%) similar to our study that shown (9%).all of pre eclamsia was wild type.

5.2. Conclusion

By the end of this study it concluded the following:

1 Activated protein C resistance (homozygous) uncommon at complicated pregnancy

2 Carrier women (heterozygous) more exposure to miscarriage

3 Miscarriage is the most common type of complicated pregnancy.

4 There is correlation between heterozygous and miscarriage and pre term labour.

3.5 Recommendations

1. Advance study should be conducted with large sample size.
2. Activated protein C resistance test should be on of screening test during pregnancy with acceptable cost.
3. Families members with complicated pregnancy should be screening for APCR.
4. APCR Test should be confirm with molecular test (PCR).

References

Appendix

6.1 References

1. Middeldorp S, Libourel EJ, Hamulyák K, Van Der Meer J, Büller HR. The risk of pregnancy‐related venous thromboembolism in women who are homozygous for factor V Leiden. British journal of haematology. 2001 May;113(2):553-5.

2. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64–7

3. Rai R, Shlebak A, Cohen H, Backos M, Holmes Z, Marriott K, Regan L. Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage. Hum Reprod 2001; 16: 961–5

4. de Visser MC, Rosendaal FR, Bertina RM. A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis. Blood 1999; 93: 1271–6

5. Graf LL, Welsh CH, Qamar Z, Marlar RA. Activated protein C resistance assay detects thrombotic risk factors other than factor V Leiden. Am J Clin Pathol 2003; 119: 52–60

6.Von Boven H H, Reitsma ph Rosendaal FR, Bay T A , scharrer I, Conrd. J. and Lane.D.A.1996.(FV R 506Q) in families with inherited anti thrombin deficiency.thrombosis and heamostasis.1960 Mar(03):417-21.

7. Zoller B, Hillarp A, Berntorp E, Dahlback B. Activated protein C resistance due to a common factor V gene mutation is a major risk factor for venous thrombosis. Annu Rev Med 1997;48:45-58.

8. Hillarp A, Zoller B, Dahlback B. Activated protein C resistance as a basis for venous thrombosis. Am J Med 1996;101:534-40

9.Esmon CT, Esmon NL, Bonniec BF, Johnson AE. Protein C activation. Meth Enzymol 1993;222:359-85

10.O'Riordan MN, Higgins JR. Haemostasis in normal and abnormal pregnancy. Best Pract Res Clin Obstet Gynaecol 2003;17:385-96.

11.Matsouka CJ. Haemostatic changes during pregnancy Haema 2005;8(Suppl 1):S68-71.

12. Faught W, Garner P, Jones J, Ivey B. Changes in protein C and protein S levels in normal pregnancy. Am J Obstet Gynecol 1995;172:147-50

13.Rai R, Backos M, Elgaddal S, et al – Factor V Leiden and recurrent miscarriage-prospective outcome of untreated pregnancies. Hum. Reprod. 2002;

14.Giorgio Mello, Elena Parretti, Luca Marozio, et al – Thrombophilia Is Significantly Associated With Severe Preeclampsia. Hypertension 2005; 46:1270

15.Nurk E, Tell GS, Refsum H, et al – Factor V Leiden, pregnancy complications and adverse outcomes: the Hordaland Homocysteine Study. QJM 2006; 99:289-298

16.Farquharson RG, Jauniaux E, Exalto N – Updated and revised nomenclature for description of early pregnancy events. Hum Reprod 2005; 20:3008-3011

17.Infante-Rivard C, Rivard GE, Guiguet M, et al – Thrombophilic polymorphisms and intrauterine growth restriction. Epidemiology 2005; 16:281-287

18. Kupferminc MJ, Many A, Bar-Am A, et al – Mid-trimester severe intrauterine growth restriction is associated with a high prevalence of thrombophilia. BJOG 2002; 109:13731376

19.Prochazka M, Lubusky M, Slavik L, et al – Frequency of selected thrombophilias in women with placental abruption. Aust NZ J Obstet Gynaecol 2007; 47:297-301

20. Hvas AM, Ingerslev J, Salvig JD – Thrombophilia risk factors are associated with intrauterine fetal death and pregnancy-related venous thromboembolism. Scand J Clin Lab Invest. 2008; 21:1-7

21. Kupferminc MJ. Thrombophilia and pregnancy. Reproductive Biology and Endocrinology. 2003 Dec;1(1):111.

22. Middeldorp S – Thrombophilia and pregnancy complications: cause or association? J Thromb Haemost 2007; 5 (Suppl. 1):276-282

23. Brenner B – Haemostatic changes in pregnancy. Thromb Res. 2004;

114(56):409-414

24.Bremme KA – Haemostatic changes in pregnancy. Best Pract Res Clin Haematol 2003; 16:153-168

25. Margareta Hellgren – Hemostasis during Normal Pregnancy and Puerperium. Semin Thromb Hemost 2003; 29:125-130

26. Mathonnet F, de Mazancourt P, Bastenaire B, et al – Activated protein C sensitivity ratio in pregnant women at delivery. Br J Haematol 1996; 92:244-246

27.Taylor FB Jr, Peer GT, Lockhart MS, Ferrell G, Esmon CT. Endothelial cell protein C receptor plays an important role in protein C activation in vivo. Blood. 2001;97:1685–1688

28. Zivelin A, Gitel S, Griffin JH, et al. Extensive venous and arterial thrombosis associated with an inhibitor to activated protein C. Blood. 1999;94:895– 901.

29.Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. Highriskofthrombosis in patients homozygous for factorV Leiden (activated protein C resistance). Blood. 1995;85:1504–1508.

30. Bloemenkamp KW, Rosendaal FR, Helmerhorst FM, Buller HR, Vandenbroucke JP. Enhancement by factorV Leiden mutation of risk of deep-veinthrombosis associated with oral contraceptives containing a third-generation progestogen. Lancet. 1995;346:1593–1596

31.Ridker PM, Hennekens CH, Selhub J, Miletich JP, Malinow MR, Stampfer MJ. Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism. Circulation. 1997;95:1777–1782

32.Vandenbroucke JP, Rosing J, Bloemenkamp KW, et al. Oral contraceptives and the risk of venous thrombosis. N Engl J Med. 2001;344:1527–1535

33.Rosendaal FR, Siscovick DS, Schwartz SM, et al. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. Blood. 1997;89:2817–2821. 27.

34. Inbal A, Freimark D, Modan B, et al. Synergistic effects of prothrombotic

polymorphisms and atherogenic factors on the risk of myocardial infarction in young males. Blood. 1999;93:2186–2190..

35 Doggen CJ, CatsVM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors: increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. Circulation. 1998;97: 1037–1041

36.Lee DH, Warkentin TE, Denomme GA, Lagrotteria DD, Kelton JG. Factor V Leiden and thrombotic complications in heparin-induced thrombocytopenia. Thromb Haemost. 1998;79:50–53

. 37.Gonzales Ordonez AJ, Medina Rodriguez JM, Martin L, AlvarezV, Coto E. The O blood group protects against venous thromboembolism in individuals with the factorV Leiden but not the prothrombin (factor II G20210A) mutation. Blood Coagul Fibrinolysis. 1999;10:303–307.

38.Robert A, Aillaud MF, Eschwege V, Randrianjohany A, Scarabin Y, JuhanVague I.ABO blood group and risk of venous thrombosis in heterozygous carriers of factorV Leiden. Thromb Haemost. 2000;83:630–631

39.Bjorgell O, Nilsson PE, Nilsson JA, Svensson PJ. Location and extent of deep vein thrombosis in patients with and without FV:R 506Q mutation. Thromb Haemost. 2000;83:648–651

40.Bounameaux H. FactorV Leiden paradox: risk of deep-veinthrombosisbut not of pulmonary embolism. Lancet. 2000;356:182–183.

41.Goldhaber S Z. Epidemiology of pulmonary embolism and deep vein thrombosis. In: Tuddenham E G D, Bloom A L, Forbes C D, Thomas D P (eds) Haemostasis and Thrombosis. Edinburgh: Churchill Livingstone, (1994) ; 1327-1333.

42Thomas DP. Pathogenesis of venous thrombosis. In: Tuddenham EGD, Bloom AL, Forbes C D, Thomas D P (eds) Haemostasis and Thrombosis. Edinburgh: Churchill Livingstone, (1994) (1991) ; 1335-1347.

43. Hirsh J. Venous Thromboembolism. In: Hoffman R et al (eds) Hematology, Basic Principles and Practice. Churchill Livingstone, 1465-(1991) ; 1484.

44. Heijboer H, Brandjes D, Büller HR et al. Deficiencies of coagulation inhibiting and fibrinolytic proteins in outpatients with deep-vein-thrombosis. New Eng J Med (1991);323, 1512-1516.

45. Malm J, Laurell M, Nilsson IM, Dahlbäck B. Thromboembolic disease - critical evaluation of laboratory investigation. Thromb Haemost (1992); 68, 7-13.

46. Pabinger I, Brücker S, Kyrle PA et al. Hereditary deficiency of antithrombin, protein C and protein S: prevalence in patients with a history of venous thrombosis and criteria for rational patient screening. Blood Coag (1992) ; Fib 3, 547-553.

47. Lane DA, Mannucci PM, Bauer KA et al. Inherited thrombophilia: part 1. Thromb Haemost (1996) ; 76, 651-662.

47. Lane DA, Mannucci PM, Bauer KA et al. Inherited thrombophilia: part 2. Thromb Haemost (1996) ; 76, 824-834.

48.Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Nat Acad Sci 90, (1993) 1004-1008.

49. Griffin JH, Evatt B, Wideman C, Fernández JA. Anticoagulant protein C pathway defective in majority of thrombophilic patients. Blood 1989-1993, 82.

50. Koster T, Rosendaal F, de Ronde H et al. Venous Thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet (1993) 342, 1503-1506.

51. Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. New Engl J Med (1994) 330, 517-522.

52. Cadroy Y, Sié P, Boneu B. Frequency of a defective response to activated protein C in patients with a history of venous thrombosis. Letters to the Editor. Blood (1994)94, 2008 -2009.

53. Bertina R M, Koeleman BPC, Koster T et al. Mutation in blood coagulation factor V associated with resistance to Activated Protein C. Nature (1994) 369, 64-67.

54.Voorberg J, Roelse J, Koopman R et al. Association of idiopathic venous thromboembolism with single point- mutation at Arg506 of Factor V. Lancet (1994) 343, 1535-1536.

55. Zöller B, Dahlbäck B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. Lancet (1994) 343, 1536-1538.

56. Zöller B, Svensson PJ, He X, Dahlbäck B. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. J Clin Invest (1994), 94, 2521-2524.

57. Ortel TL, Kane WH. Factor V. In: High KA, Roberts HR (eds) Molecular basis of thrombosis and hemostasis. New York: Marcel Dekker (1995) , 119-146.

58. Tuddenham EGD. Factor VIII. In: High KA, Roberts HR (eds) Molecular basis of thrombosis and hemostasis. New York: Marcel Dekker (1995), 167-195.

59. Mann KG, Jenny RJ, Krishnaswamy S. Cofactor proteins in the assembly and24 expression of blood clotting enzyme complexes. Annu Rev Biochem (1988) 57, 915-956.

Hoyer LW. Hemophilia A. New Engl J Med 330, 38-47 (1994). .60

61. Dahlbäck B. The protein C anticoagulant system: inherited defects as a basis for venous thrombosis. Thromb Res (1988) 77, 1-43 .

62. Kalafatis M, Rand MD, Mann KG. The mechanism of inactivation of human factor V and human factor Va by activated protein C. J Biol Chem (1994) 269, 31869-31880.

63. Kalafatis M, Bertina RM, Rand MD, Mann KG. Characterization of the molecular defect in factor VR506Q. J Biol Chem (1995) 270, 4053-4057 .

64. Heeb MJ, Kojima Y, Greengard JS, Griffin JH. Activated protein C resistance:

molecular mechanism based on studies using purified Gln506factor V.Blood (1995) 85, 3405-3411.

65. Griffin JH, Heeb MJ, Kojima Y et al. Activated protein C resistance: molecular mechanism. Thromb Haemost (1995) 74, 444-448.

.66. Aparicio C, Dahlbäck B. Molecular mechanisms of activated protein C resistance. Properties of factor V isolated from an individual with homozygosity for the Arg506 to Gln mutation in the factor V gene. Biochem J (1996) 313, 467-472.

67. Dahlbäck B, Hildebrand B. Inherited resistance to activated protein C is corrected by anticoagulant cofactor activity found to be a property of factor V. Proc Nat Acad Sci 91, (1994) 1396-1400.

68. Sun X, Evatt B, Griffin JH. Blood coagulation factor Va abnormality associated with resistance to activated protein C in venous thrombophilia. Blood (1994)11, 3120-3125.

69. De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. Blood (1996) 87, 3531-3544

70. Johansson K, Lindberg K, Menschik M, Rosén S. Evaluation of a kit for detection of resistance in plasma towards activated protein C activity in a modified APTT assay. Thromb Haemost (1993) 69, 1120.

71. Olson ST. Björk I. Regulation of thrombin by antithrombin and heparin cofactor II. In: Berliner LJ (ed) Thrombin: structure and function. New York: Plenum Press(1992) 159-217,.

72. Rosén S, Johansson K, Lindberg K, Dahlbäck B. Multicenter evaluation of a kit for activated protein C resistance on various coagulation instruments using plasmas from healthy individuals. Thromb Haemost (1994) 72, 255-260.

73. Andersson NE, Lindberg K, Johansson K et al. Methodological considerations on the determination of the APC response in plasma. Hämostatisches Gleichgewicht(1995) 22, 80-82.

74. De Stefano V, Paciaroni K, Mastrangelo S et al. Instrument effect on the

activated protein C resistance plasma assay performed by a commercial Kit.Thromb Haemost (1996) 75, 752-756.

75. Hall CM, Andersson NE, Andras M et al. Rapid and reliable detection of FV:Q506 in plasma from heparin- and OAC-treated patients using the Coatest® APC™ Resistance V assay. Blood 10, suppl 1, (1996) 172a.

77. Zöller B, Dahlbäck B. Resistance to activated protein C caused by a factor V gene mutation. Current Opinion in Hematology(1995) 2, 358-364

78.Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet(1995) 346, 1133-1134.

79. Arruda VR, von Suben PM, Soares MCP et al. Very low incidence of Arg506-Gln mutation in the factor V gene among the Amazonian Indians and the Brazilian black population.Thromb Haemost (1996)75, 860-861.

80. Soubrier F, Fery I, Verdy E et al. The frequency of the factor V gene R506Q mutation varies between regions of France. Nouv Rev Fr Hematol (1995) 37, 175.

81. Emmerich J, Poirier O, Evans A et al. Myocardial Infarction, Arg506 to Gln factor V mutation, and activated protein C resistance. Letters to the Editor. Lancet (1995)345, 321.

82. Cox MJ, Rees DC, Martinson JJ, Klegg JB. Evidence for a single origin of factor V Leiden. Br J Haem 92, (1996) 1022-1025.

83. Gou D, Naipai A and Reitsma PH. World distribution of factor V Leiden mutation. Letters to the Editor. Lancet (1995) 347, 59 X.

84. Aillaud MF, Suucco E, Alessi MC et al. Resistance to activated protein C - diagnostic strategy in a laboratory of haemostasis. Letter to the Editor. Thromb Haemost (1995)74, 1197-1207.

85. Zehnder JL, Benson RC. Sensitivity and specificity of the APC resistance assay in detection of individuals with factor V Leiden. Am J Clin Pathol(1996) 106:1, 107-111.

86. Dahlbäck B. Resistance to activated protein C, the Arg506 to Gln mutation in the factor V gene, and venous thrombosis. Functional tests and DNA-based assays, pros and cons. Thromb Haemost (1995)73, 739-742.

87. Florell SR, Rodgers III GM. Utilization of testing for activated protein C resistance in a reference laboratory. Am J Clin Pathol (1996)106, 248-252.

88. Sakata T, Kario K, Katayama Y et al. Clinical significance of activated protein C resistance as a potential marker for hypercoagulable state.Thromb Res(1996) 82, 235-244.

89. De Lucia D, de Blasio G, Belli A et al. High prevalence of activated protein C resistance in patients with systemic sclerosis. Int J Clin Lab Res (1995) 25, 226-227.

90. Rai R, Regan L, Hadley E et al. Second-trimester pregnancy loss is associated with activated protein C resistance. Br J Haem (1996) 92, 489-490.

91. Fisher M, Fernández JA, Ameriso SF et al. Activated protein C resistance in ischemic stroke not due to factor V Arginine506>Glutamine mutation.Stroke(1996) 27, 1163-1166.

92. Van der Bom JG, Bots ML, Haverkate F et al. Reduced response to activated protein C is associated with increased risk for cerebrovascular disease. Ann Intern Med(1996) 125, 265-269.

93. Ouriel K, Green RM, De Weese JA, Cimino C. Activated protein C resistance: prevalence and implications in peripheral vascular disease. J Vasc Res(1996) 23, 46-52.

94. Schlit AF, Col-de Beys C, Moriau M, Lavenne-Pardonge E. Acquired activated protein C resistance in pregnancy. Thromb Res(1996) 84, 203-206

95.Olivieri O, Friso S, Manzato F et al. Resistance to activated protein C, associated with oral contraceptives use; effect of formulations, duration of assumption, and doses of oestro-progestins. Contraception(1996) 54, 149-152.

96. a. Østerud B, Robertsen R, Åsvang GB, Thijssen F. Resistance to activated

protein C is reduced in women using oral contraceptives. Blood Coag Fibr(1994) 5, 853-854.

97. b. Girolami A, Paulo S, Bruno G, Luigi S. Oral contraceptive therapy causes an increased and not a decreased resistance to APC and Authors' reply by Østerud. Letters to the Editor. Blood Coag Fibr (1995)6, 143 -144.

98.Bauer KA. Management of patients with hereditary defects predisposing to thrombosis including pregnant women. Thromb Haemost (1995)74, 94-100.

99. De Stefano V, Mastrangelo S, Paciaroni K et al. Thrombotic risk during pregnancy and puerperium in women with APC resistance- effective subcutaneous heparin prophylaxis in a pregnant patient. Letters to the editor. Thromb Haemost(1995) 793-794.

100. Bourjeily G, Paidas M, Khalil H, Rosene-Montella K, Rodger M. Pulmonary embolism during pregnancy. Lancer 2009; 375(suppl 9713):500–512

101. Zivelin A, Mor-Cohen R, Kovalsky V, Kornbrot N, Conrad J, and Peyvi. Fetal Prothrombin 20210G>A is ancestral prothrombotic mutation that occurred in white approximately 24,000 years ago. Blood 2006; 107:4666–4668.

102. Ľubica Hammerová1, Ján Chabada1, Juraj Drobný1, Angelika Bátorová 2 Comenius University, Faculty of Medicine University Hospital, Bratislava, Slovakia: 1st Department of Gynaecology and Obstetrics1.The National Haemophilia Centre, Institute of Haematology and Blood Transfusion, University Hospital, Bratislava, Slovakia 2.

103.Wilmer M, Stocker C, Bühler B, Conell B, Calatzis A. Improved distinction of factor V wild-type and factor V Leiden using a novel prothrombin-based activated protein C resistance assay. American journal of clinical pathology. 2015;122(6):836-42.

104. Adcock DM, Kressin DC, Marter RA.Minimumspecimen volume requirements forroutine coagulation testing: dependence on citrate concentration. American journal of clinical pathology.1998 May 1.109(5):595-9.

105. Mekaj Y, Zhubi B, Hoxha H, Belegu R, Mekaj A, Miftari E, Belegu M. Prevalence of resistence to activated protein C (APC-resistance) in blood donors in Kosovo. Bosnian journal of basic medical sciences. 2009 Nov;9(4):329.

Questionnaire

University of Shendi

Faculty of post graduate studies

Faculty of medical laboratory sciences

Role of Factor V Leidin and hereditary anticoagulant deficiency in Vascular complication of pregnancy

S.Number: ……

Hospital: ……..

(1) Lab NO ……...

(2) Age …….

(3) BMI …….

(4) Party ……

(5) Gestational age ……

(6) Race …….

(7) Region …….

(8) Family history of pregnancy complication ……..

(9) Relation degree ……

(10) Diagnosis:

Preeclampsia

IUGR

PTL

Stillbirth

Miscarriage

Chorionamnioitis

(11) History of disease:-

DM

Hypertension

Bleeding

(12) Result: ……..