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Evaluation of Factor V Leiden Mutation among Pregnant Women With Preeclampsia

تقيم وجود الطفرة الجينية في عامل التجلط رقم 5 وسط النساء الحوامل المصابات بتسمم الحمل

A Dissertation Submitted in Partial Fulfillment for Requirements of M.Sc. Degree

In Medical Laboratory Sciences (Haematology and Immunohematology)

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بسم الله الرحمن الرحيم

الآيـــــة

قال تعالى:

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

صدق الله العظيم

سورة يوسف (الآية : 76)

DEDICATION

I dedicate this work to:

My darling parents who are supporting me and encouraging me

to success.

My brothers and my friends and my all family .

My colleagues who gave me the possibility of completing this dissertation

Everyone who has helped me to learn new things and to reach this level of knowledge

.

Acknowledgment

First of all my gratitude and thanks to our Almighty Allah, most gracious and most merciful, whom gave me the serenity , means of strength and patience to finish this work.

With grateful appreciation acknowledgment the effort of my supervisor Dr.KawtharAbdelgaleil Mohammed Salih for her supervision , unlimited patience , generous supporting and guidance.

I would like to express my gratitude and sincers thanks to staff of OmdurmanMaternityHospital for the help during samples collection . As my thanks extended to all volunteers both pregnant and non-pregnant women who contributed to complete this research.

Finally , my deep gratitude goes to the members of MedicalLaboratoryCollege in Sudan University of Science and Technology for their motivation during this study.

Abstract

This is a case control study ,carried in Khartoum state during the period from April to March 2019 , aimed to evaluate of factor V Leiden mutation among pregnant women with preeclampsia in Omdurman Maternity Hospitals. The study included 100 Sudanese females 50 pregnant women with preeclampsia and 50 non pregnant women , their age ranged 18 – 49years.

All volunteers were verbally informed about the study and their consent for participation was obtained.3 ml of EDTA venous blood sample was collected from each volunteer. Polymerase Chain Reaction (eppendorf, master cycler) was used for detection of factor V Leiden mutation .

The data collected consist of demographic data of women included age , education level , history of chronic disease , number of gravidity , history of thrombosis , and history of abortion.

100% of women were not have mutantion in factor V. Data analyze by using SPSS version 16(Statistical Package for Social Sciences).

Age divided into three age groups (< 20) ,(20-35),and (>35) years, the most frequent one was 20-35 years , while the least one was < 20 years in case group. As like control group, with mean and STD (29.1$\pm$ 6.1) years.

In case group Regarding to the educational level , 6% of women had illiterate ,36% had higher education level. Healthy women were highest frequency compared with women have hypertension (80% , 20%) respectively, most of them (18%) use Nifedipine and 2% use Amilo5. According to gravidity high frequency was primy gravidity 32%, followed by secondy gravidity and grand multi gravity 28% and then the multi gravidity 12% .

Eighty six of women not have thrombosis history but 14 % of them have history of thrombosis , regard to the abortion 40% have history of abortion and 60 % not have history , high frequency of abortion number is one time 22%, two time 14% and three time 4% .

The results showed absence of gene mutation in both case and control, with no statistically significant difference in factor V between pregnant and non pregnant women.

Also there was no significant association between mutation and demographic data , as well as mean of age between cases and control *p.value* (p=0.56).

مستخلص الدراسة

هذه دراسة حاله أجريت في ولايه الخرطوم في الفترة من أبريل إلى مارس 2019م وهدفت لتقييم وجود طفرة جينية في عامل التجلط رقم (5) عند النساء المصابات بتسمم الحمل في مستشفى الولادة بأمدرمان . وشملت هذه الدراسة 100 امرأة سودانيه ، 50 منهن حوامل مصابات بتسمم الحمل و 50 منهن غير حوامل (أعمارهن بين 18 -49 سنه).

أطلعت كل المشاركات شفهيا عن الدراسه واخذت موافقتهن على المشاركه . جمعت 3 مل من الدم الوريدي في عامل التجلط (ثنائي أمين الإيثيلين رباعي حمض الأسيتيك) تم جمعها من كل متبرعه. واستخدم جهاز تفاعل البلمره المتسلسل (eppendorf, master cycler) لإكتشاف وجود طفرة جينية في عامل التجلط رقم (5).

جمعت البيانات االسكانيه عن طريق استبيان وتضم العمر،المستوى التعليمي ،التاريخ الطبي للامراض المزمنه ، عدد الولادات ، التاريخ الطبي للجلطات ، التاريخ الطبي للاجهاض والمتابعه المنتظمه مع الطبيب اثناء فتره الحمل .

100 % من النساء ليس لديهن طفرة جينية في عامل التجلط رقم (5) . حللت النتائج باستخدام برنامج الحزم الاحصائيه إصدار 16 .

قسم العمر الى ثلاث فئات عمريه (>20) ،(20-35) ، (< 35) سنه اكثرهم تكرارا الفئه من 20-35 سنه وأقلهم تكرارا الفئه العمريه 20> في الحوامل منهن وكذلك في غير الحوامل . بمتوسط وإنحراف معياري (29.1 $\pm$ 6.1) سنه.

في مجموعه النساء الحوامل فيما يتعلق بالمستوى التعليمي ، 6% منهن غير متعلمات ، 36% لديهن مستوى تعليم عالي . النساء السليمات هن الاكثر تكرارا مقارنه بالنساء المصابات بإرتفاع ضغط الدم (80% ،20%) على التوالي .معظم المصابات (18%) يستخدمن عقار Nifedipine و 2% يستخدمن عقار Amilo5.

وفقا لعدد مرات الحمل الأكثر تكرارا حمل مره واحده 32%، يتبعها مرتان و ثلاثه مرات 28% ، واخيرا اكثر من ثلاثه مرات 12%.

سته وثمانون من النساء ليس لديهن تاريخ مرضي للإصابه بالجلطات لكن 14% منهن لديهن تاريخ مرضي للجلطات ، فيما يتعلق بالإجهاض 40% منهن تعرضن للإجهاض و 60% منهن لم يتعرضن ، وعدد مرات الإجهاض الأكثر تكرارا مره واحدة 22%، مرتان 14% و ثلاثه مرات 4%.

اوضحت النتائج غياب الطفره الجينيه في كلا المجموعتين (حوامل وغير حوامل)مع عدم وجود فرق ذو دلاله احصائيه في عامل التجلط رقم (5) بين الحوامل وغير الحوامل .

و ايضا انه لا يوجد علاقه بين الطفره الجينيه و البيانات الديموغرافيه وكذلك في متوسط الأعمار بين النساء الحوامل والغير حوامل(القيمه المعنويه 0.56).

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Abbreviations	full name
APC	Activated protein C
APTT	Activated thromboplastin time
ARMS	Amplification refractory mutation system
ATIII	Antithrombin III
C	Common
Ca^{++}	Calcium
DVT	Deep vein thrombosis
FDP	Fibrin degradation product
FV	Factor 5
FVa	Factor 5 active
FVL	Factor 5 Leiden
GP	Glycoprotein
HRT	Hormone replacement treatment
IUGR	Intrauterine growth restriction
M	Mutant
N	Normal
OC	Oral contraceptive
PE	Preeclampsia
PC	Protein C
PS	protein S
PT	Prothrombin time

TFPI	Tissue factor pathway inhibitor
TPA	Tissue plasminogen activator
UPA	Urokinaselike plasminogen activator
VWFVon willebrand factor	

Chapter one

Introduction and literature review

1.1 Introduction:

1.1.1 Coagulation

Coagulation (also known as clotting) is the process by which blood changes from a liquid to a gel , forming a blood clot. It potentially result in hemostasis , the cessation of blood loss from a damaged vessel ,following by repair. The mechanism of coagulation involve activation, adhesion ,and aggregation of platelets along with deposition and maturation of fibrin (Lillicrap *et al*.,2009).

The several important functions of hemostasis included : maintain blood in a fluid state while it remains circulating within the vascular system, also arrest bleeding at the site of injury or blood loss by formation of a haemostatic plug, also limit process to the vicinity of the damage ,and to ensure the eventual removal of the plug whilst healing is completed. Normal physiology thus constitutes a delicate balance between these conflicting tendencies, and a deficiency or exaggeration of any one may lead to either thrombosis or hemorrhage (Bain *et al.*,2017).

There are different stages and phases in hemostasis process, which have involved different cell lines and different proteins (soluble in idle status) of blood. The final result is the formation of a red/fibrin mesh (insoluble protein in the blood) inside it encompassed blood cells (platelets, erythrocytes). This grid/mesh acts as a barrier and prevents the loss of blood vessel injury by until the vascular tree is repaired (Blanco *et al.*,2014).

1.1.2 Factor V and factor V Leiden:-

Factor V (F V), is one of coagulation factor .which was discovered by Paul Owren in 1943 , has proved to be an important regulator of the hemostatic balance with both procoagulant and anticoagulant properties(Hiltunen and Rautanenm ,2011)**.**

The point mutation causes the replacement of amino acid Arg to Gln at the site 506 in factor V resulting in the inadequate inactivation of mutated F Va. The mutation was named as factor V Leiden (FV Leiden) (Bagheri *et al*.,2011).

1.1.3 preeclampsia :-

Pre-eclampsia and eclampsia are two hypertensive disorders of pregnancy, considered major causes of maternal and perinatal death worldwide. It is a multisystemic disease characterized by the development of hypertension after 20 weeks of gestation, with the presence of proteinuria or, in its absence, of sign or symptoms indicative of target organ injury (Shamsi *et al*.,2014).Eclampsia represents with consequence of brain injuries. So correct diagnosis and classification are essential. Since the therapies for the mild and severe forms of preeclampsia are different (Peres *et al*.,2018).

1.1.4 Factor V Leiden and preeclampsia:-

Factor V Leiden (FV Leiden), is the most common known inherited thrombophilia in Caucasians . Due to this mutation, activated F V (F Va) are improperly cleaved and neutralized by activated protein C (APC). This phenomenon is named APC resistance and it leads to enhanced production of thrombin (Laczmanski *et al*.,2013).Thrombophilia has been associated, not only with venous thrombosis, but also with many specific pregnancy complications (pregnancy loss, preeclampsia, intrauterine growth restriction (IUGR),placental abruption)(Hiltunen and Rautanenm,2011).

1.2 Literature review:

1.2.1 Hemostasis:-

Is the process by which bleeding is stopped after an injury by the formation of a clot, and at the same time, maintaining blood in a fluid state elsewhere. It has three major steps: 1) vasoconstriction, 2)temporary blockage of a break by a platelets , and 3) blood coagulation , or formation of fibrin clot,these processes seal the hole until tissue are repaired (Bhaskar *et al*.,2016).

It is divided into two principal phases. The first, defined as primary hemostasis, involves the platelet-vessel interplay, whilst the second, defined as secondary hemostasis, mainly involves coagulation factors, damaged cells and platelet surfaces, coagulation cascade rapidly develops (Gale,2011). The activation and amplification of the coagulation cascade is finely modulated by the activity of several physiological inhibitors. Once bleeding has been efficiently stopped by blood clot formation, dissolution of the thrombus is essential to restore vessel permeability. This process, known as fibrinolysis, also develops through coordinate action of a vast array of proteins and enzymes (Lippi and Favaloro,2018).

1.2.2 Primary hemostasis:-

Platelets are small a nuclear cell fragments that bud off from megakaryocytes, specialized large blood cells that originate in the bone marrow they are present at 150 to 400 million per milliliter of blood and circulate for about ten days (Hoffbrand *et al*.,2006).In a healthy blood vessel, and under normal blood flow, platelets do not adhere to surfaces or aggregate with each other. However, in the event of injury platelets are exposed to subendothelial matrix, and adhesion and activation of platelets begins. Multiple receptors on the surface of platelets are involved in these adhesive interactions, and these receptors are targeted by multiple adhesive proteins(Gale,2011).

1.2.2.1 Platelets function:-

The main steps in platelet functions are adhesion, activation with shape change and aggregation.When the vessel wall is damaged, the subendothelial structures, including basement membrane, collagen and microfibrils, are exposed (Bain *et al*.,2017).VWF binds to collagen and microfibrils and then captures platelets via initial binding to platelet GPIb, resulting in an initial monolayer of adherent platelets. Binding via GPIb initiates activation of the platelet via a G-protein mechanism. Once activated, platelets

immediately change shape from a disc to a sphere with numerous projecting pseudopods. After adhesion of a single layer of platelets to the exposed subendothelium, and stick to one another to form aggregates. Fibrinogen, fibronectin, further VWF released from platelets and the glycoprotein IbIX and IIbIIIa complexes are essential at this stage to increase the cell-to-cell contact and facilitate aggregation(Ciesla,2007).

1.2.2.2 Role of platelets in haemostasis:-

Adhesion and aggregation forming the primary haemostatic plug, Clot retraction, Release of platelet activating and procoagulant molecules,Provision of a procoagulant surface for the reactions of the coagulation system (Munker *et al*.,2007).

1.2.3 Secondary hemostasis:-

Secondary hemostasis involves a series of blood protein reactions through a cascade-like process that concludes with the formation of an insoluble fibrin clot. This system involves multiple enzymes and several cofactors as well as inhibitors to keep the system in balance. Coagulation factors are produced in the liver, except for factor VIII, which is believed to be produced in the endothelial cells.When the factors are in a precursor form, the enzyme or zymogen is converted to an active enzyme or a protease (Beutler *et al*.,2010).

The initiation of clotting begins with the activation of two enzymatic pathways that will ultimately lead to fibrin formation: the intrinsic and extrinsic pathways. Both pathways are necessary for fibrin formation, but their activating factors are different. Intrinsic activation occurs by trauma within the vascular system, such as exposed endothelium. This system is slower and yet more important versus the extrinsic pathway, which is initiated by an external trauma, such as a clot and occurs quickly (Lippi and Favaloro,2018).

1.2.3.1 Phases of Coagulation:-

Coagulation can be divided into three separate phases: *1*) an initiation phase, in which low amounts of active coagulant factors are generated; *2*) an amplification phase, in which the level of active coagulation factors is boosted; and *3*) a propagation phase, in which coagulation factors bind to highly procoagulant membranes of activated platelets and fibrin clots are formed andextrinsic pathway is still widely used (Versteeg *et al*.,2013).

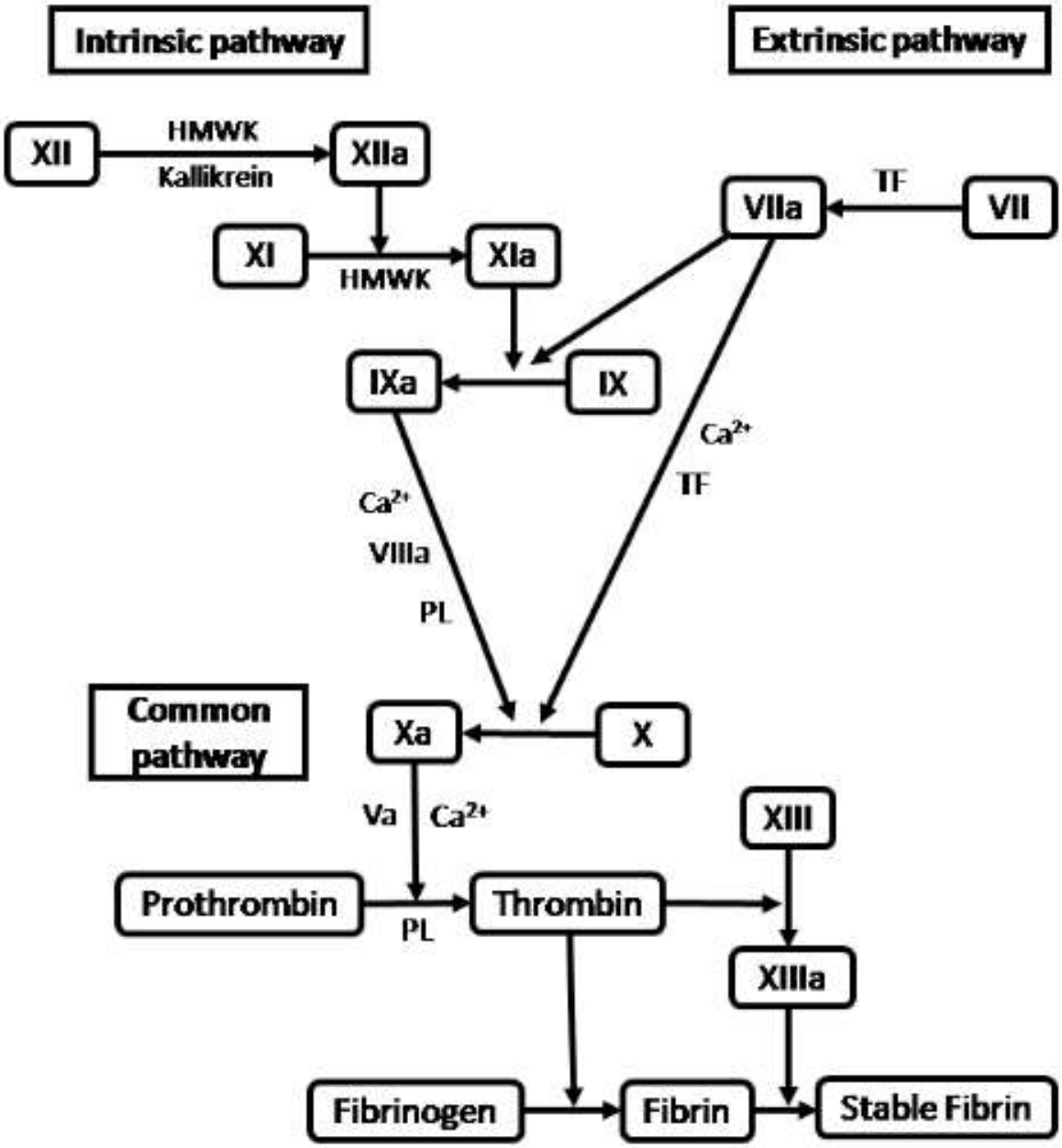

Figure 1. The cascade model of haemostasisCa2+- Calcium ion, HMWK – High molecular weight kininogen. TF – Tissue factor(Bhaskar *et al*.,2016).

1.2.4 Regulation of blood coagulation:-

The regulation of blood coagulation to ensure the action of thrombin is limited to the site of injury and it regulate by : Antithrombin inactivates serine proteases, principally factor Xa and thrombin. Heparin activates antithrombin,α2 macroglobulins, α2 antiplasmin, α2 antitrypsin and heparin cofactor II also inhibit circulating serine proteases, Proteins C and S are vitamin K-dependent proteins made in the liver (Munker *et al*.,2007). Protein C is activated via a thrombin–thrombomodulin complex and, like protein S, inhibits coagulation by inactivating factors Va and VIIIa; it also enhances fibrinolysis by inactivating the tissue plasmogen activator (TPA) inhibitor, Tissue factor pathway inhibitor (TFPI) inhibits the coagulation pathway by inhibiting factors VIIa and Xa (Mehta and Hoffbrand,2005).

Antithrombin is the most important of these inhibitors,the second most (clinically) important natural anticoagulant system is that of PS-PC (Lippi and Favaloro,2018).

1.2.5 Fibrinolysis :-

Fibrinolysis is the process in it plasmin degraded the fibrin. Following injury, TPAand urokinase-like plasminogen activator (UPA) released from damaged or activated cells, or exogenous agents, e.g. streptokinase, or therapeutic TPA or UPA,it activate plasminogen and convert it to plasmin. It digests fibrin (or fibrinogen) into fibrin degradation products (FDPs) and also degrades factors V and VII. Free plasmin is inactivated by plasma α2 antiplasmin and α2 macroglobulin (Mehta and Hoffbrand,2005).

1.2.6 Factor V:-

The gene of F V is on the chromosome 1 (1q23), and this single-chained glycoprotein of 2,196 amino acids is synthesized in the liver(LaBonte,2014).

Of the total F V, 20% is stored in platelet α-granules, the rest circulates in plasma . The F V in platelets is of plasma origin, but it is already modified in platelets by partial proteolysis, giving it considerable F Xa-cofactor activity . This seems to be an efficient way to ensure that this important factor is immediately present at the site of vessel wall injury and ready to function (Hoffbrand *et al*.,2006).

F V is activated by F Xa or thrombin to F Va by the cleavage of three peptide bonds (Arg709, Arg1018, Arg1545) . The inactivation of F Va is mediated through APC, which cleaves the F Va at the sites Arg506, Arg306, and Arg679. The Arg506 is the preferred site for proteolysis, but protected by F Xa in prothrombinase complex when

coagulation is in process. However, protein S accelerates the slower proteolysis at the site Arg306 and helps APC to reach the Arg506 site . After cleavage at the site Arg506, F Va still has partial procoagulant activity, which is abolished when the Arg306 and Arg679 peptide bonds are cleaved (Segers *et al*.,2012)**.**

1.2.7 Factor V Leiden:-

Discovered by Dahlback and his colleagues in 1994,and Bertina her colleagues In June 1994, they published a paper showing that a single G to A substitution at the nucleotide position 1691 in the factor V gene was associated with APC resistance . The point mutation causes the replacement of amino acid Arg to Gln at the site 506 in factor V resulting in the inadequate inactivation of mutated F Va. The mutation was named as factor V Leiden (FV Leiden) (Hiltunen and Rautanenm,2011)**.**

In 1998, two other rare factor V mutations were found: arginine (Arg306) to glycine (FV Hong-Kong) or threonine (FV Cambridge) (Hammerová *et al*.,2011).

1.2.7.1 Facor V Leiden and DVT:-

DVT is a common vascular condition that arises from the formation of a blood clot within the deep veins of the circulatory system. The pathogenesis of venous thrombosis is including acquired and genetic risks factors. Genetic risk factors include the deficiency of protein C, protein S, antithrombin and mutations of factor V Leiden and Prothrombin gene (Yousif *et al*.,2017).

Factor V Leiden is the most common genetic risk factor for VTE, found in 20–25% of patients with VTE and 50% of patients with familial thrombophilia. Testing for Factor V Leiden is now one of the most frequently ordered molecular genetic tests(Dajani *et al*.,2012).

The FV Leiden allele is associated with a hypercoagulable state , the mutation renders Factor Va resistant to cleavage by activated protein C (APC), which results in increased thrombin generation and higher levels of prothrombin fragment . Mutant proteins confer a 5- to 10-fold greater risk of developing DVT in heterozygous individuals and a 50- to 100-fold higher risk in homozygotes compared with normal individuals (Ibrahim *et al*.,2018).

1.2.7.2 Prevalence :-

Factor V Leiden is the most common inherited form of inherited thrombophilia, accounting for 40–50% of cases. Heterozygosity for Factor V Leiden occurs in 3–8%

of the general US and European populations. The highest heterozygosity rate is found in Europe; the mutation is extremely rare in Asian, African, and indigenous Australian populations. Within Europe, the prevalence varies from 10 to 15% in southern Sweden and Greece to 2–3% in Italy and Spain. Mutation is found in 3.8% of individuals in France, but the frequency ranges from 1.3% in southwestern regions to 7.1% in northeastern France.9 In the United States, the prevalence reflects the world distribution of the mutation (Nevalainen *et al*.,2018).

The frequency of homozygosity for Factor V Leiden in white populations is approximately 1 in 5000. Haplotype analysis of the Factor V gene strongly suggests that the mutation was a single event that occurred 20,000–30,000 years ago, after the evolutionary separation of whites from Asians and Africans (Beck,2009).

1.2.7.3 Prevention of pregnancy complications:-

American College of Chest Physicians and obstetric consensus guidelines and expert opinion do not routinely recommend antithrombotic therapy for women with Factor V Leiden and pregnancy loss because of a lack of sufficient evidence confirming benefit(Hammerová *et al*.,2011).

Antithrombotic prophylaxis may be considered in selected women with Factor V Leiden and unexplained recurrent or late pregnancy loss after an informed discussion of the risks and the limited data suggesting benefit. Assessment of the maternal thrombotic risk during pregnancy should be incorporated into the decision regarding prophylaxis. There is no evidence that antithrombotic therapy reduces the risk of preeclampsia or other pregnancy complications in women with thrombophilia including Factor V Leiden (Kujovich,2011).

1.2.8 Preeclampsia:-

preeclampsia is one of the most common complications of pregnancy. which, associated with hypertension, edema, and proteinuria, affects 5% to 10% of pregnancies worldwide and entails severe consequences for both the mother and the fetus.1–3 Preeclampsia presents as either a mild or severe condition, distinguished most often by severity of hypertension and proteinuria symptoms (Lai *et al*.,2011).

Hypertension is the most prevalent maternal complication worldwide, and it is associated with a significant morbidity and mortality of the mother and fetus, hypertension is the second largest cause of direct maternal death worldwide (14% of the total) and it is estimated that 192 people die every day because of hypertensive disorders in pregnancy(Peres *et al.*,2018).

Preeclampsia causes has not yet been elucidated and is most probably heterogenic . One possible cause is a maternal pro-thrombogenic state, leading to mal-adaptation of the spiral arteries in early pregnancy and placental thrombosis and infarction in mid- and late pregnancy. A pro-thrombogenic state can be caused by inherited or acquired thrombophilia (McDermott *et al*.,2017).

An explanation of these conflicting results that preeclampsia is a heterogenic disease with different phenotypes. preeclampsia be classified as mild or severe, it may also be complicated by hemolysis, elevated liver enzymes or low platelets (HELLP-syndrome) and/or intrauterine growth restriction (IUGR) and/or early onset preeclampsia. Histological examination of placentas of pregnancies complicated by preeclampsia may show extensive infarction, but also no abnormal findings at all . early preeclampsia has another pathophysiological basis than late preeclampsia (Berks *et al*.,2015).

1.2.8.1 Etiology of preeclampsia:-

Preeclampsia is usually described as a two-stage syndrome . The first stage is characterized by shallow invasion of fetal trophoblast cells into the decidua and inadequate modification of the spiral arteries. This leads to uneven blood flow to the placenta, and thus to placental stress (Kenny and Myers,2017).

The damaged placenta lead to releases a number of factors and placental debris. As result of these, fragments of syncytiotrophoblast cells, basal membrane, microparticles, microRNA, and fetal DNA have been detected in the maternal circulation where they cause inflammation and endothelial injuries(Mecinaj,2014).

The second stage of PE is characterized by the maternal disease, presenting with elevated blood pressure and proteinuria. Some suggestion that a factor, X, links stages 1 and 2, but more likely there are several factors contributing to the PE etiology. Which contribute to the maternal inflammation, endothelial damage, and clinical findings of PE—elevated blood pressure and proteinuria(Anderson and Ulrik,2015).

1.2.8.2 Risk factors of preeclampsia:-

Primipaternity,Chronic hypertension,Pregnancy after donor insemination,Renal disease,Pregnancy after oocyte/embryo donation,Obesity and insulinresistance,Extremes of maternal age,Pregestational diabetes mellitus, Multifetal gestation ,Maternal infections,Preeclampsia in previous pregnancy, and Hydropic degeneration of the placenta (Hammerova *et al*.,2011).

1.2.9 Factor V Leiden and preeclampsia :-

The predisposition to inherited thrombophilia, during pregnancy increases because the changes associated with it in some coagulation factors cause increase in resistance to activated protein C (PrC) during second and third trimesters and decrease in activity of protein S (PrS) because of estrogen effects; also, factors 2, 7, 8, 9, and 10 increase (Dehkordi *et al*.,2014).

All these lead to increase in coagulability in healthy women. Therefore, an individual with inherited thrombophilia is at high risk of thromboembolic problems during pregnancy, which is often followed by repeated abortions, preeclampsia, stillbirth, intrauterine growth restriction (IUGR), detached pair, increase in cardiovascular events, deep vein thrombosis (DVT) and pulmonary embolism (Nevalainen *et al*.,2018).

The most prevalent factors causing genetic thrombophilia, PrS deficiency, PrC deficiency, factor V Leiden (FVL) mutation, and antithrombin III (ATIII) deficiency. In several studies in Turkey, Canada, Italy and in a study by Lockwood and Wendel, inherited thrombophilia was identified as a risk factor for pregnancy complications such as repeated abortions and preeclampsia (Dehkordi *et al.*,2014).

Abnormal FV molecule which undergoes a slower inactivation persists longer in the circulation system, resulting in an increased risk of clotting .Thrombophilia risk is higher when the mutation co-exists with: obesity, a hormone replacement therapy (HRT), oral contraceptives (OC), a pregnancy,a surgery or smoking. Mutations of the FV Leiden (FVL) are inherited in an autosomal dominant manner with incomplete penetration (Łaczmanski *et al.*,2013).

Dysfunction of the maternal vascular endothelium and abnormal placentation are pathophysiological mechanisms predispose that pregnant women to preeclampsia . The thrombotic tendency in some women with preeclampsia may be associated with the development of preeclampsia through abnormal placentation (Babeker *et al*.,2016).

Genetic factors may play a role in the pathophysiology of preeclampsia. Inherited thrombophilias. However, the combinedeffects of both FVL and prothrombin G20210A mutationson a hypercoagulable state in pregnancy which could affectthe increased risk of preeclampsia has been suggeste (Khosravi *et al*.,2012).

1.2.10 Previous studies :-

In (2014) Elbaz and his colleges conducted Comparative Study on the Effect of Factor V Leiden and Prothrombin gene Polymorphism in Preeclampsic Cases, and concluded that Heterozygous AG genotype showed a significant high frequency among preeclampsic patients (20.7%) compared to controls (4.0%), (OR 6.2, P= 0.006) (Elbaz *et al.*,2014).

In (2011) Hammerovaand his colleges study of factor V Leiden mutation and its impact on pregnancy complications, and found that Carriership of the factor V Leiden mutation did not affect the incidence of preeclampsia (Hammerová *et al*.,2011).

In (2012) Karimi and his colleges study Evaluation the frequency of factor V Leiden mutation in pregnant women with preeclampsia syndrome in an Iranian population, and found that In total, 17(8.6%) of cases and 2(1%) of controls showed the factor V Leiden mutation. The incidence of factor V Leiden was typically higher in preeclamptic women than control group (OR: 9.34 %95 CI: 2.12-41.01) (Karimi *et al*.,2011).

In (2014) Saghafi and her colleges study Evaluation of selected thrombotic factors among pregnant women with preeclampsia and normal pregnant women, and show that the number of patients with abnormal factor V Leiden and protein C was significantly higher in case group than in the control group ($p<0.01$ respectively) (Saghafi *et al*.,2014).

In (2018) Nevalainen and her colleges study to show if the Placenta-mediated pregnancy complications are associated with fetal or paternal Factor V Leiden mutation or not and concluded that Fetal or paternal factor V Leiden mutation is not associated with severe placenta-mediated pregnancy complications(Nevalainen *et al*.,2018).

In (2011) Bagheri and his colleges study of Factor V Leiden G1691A and factor II G20210A point mutationsand pregnancy in North-West of Iran and found that FV Leiden G1691A mutation was not found in the studied cases and controls, that is, all of the cases and the controls had normal FV Leiden 1691GG genotype (Bagheri *et al.*,2011).

In (2016) salari and his colleges studied the association between preeclampsia and defined polymorphisms in prothrombin and coagulation factor v genes, and show that no significant difference when compare of preeclamptic and control group for single nucelotid polymorphism (G1691A) (Salari *et al*.,2016) .

In (2016) Shalaby and his colleges study of Thrombophilia gene mutations in relation to recurrent miscarriage, and found that Factor V Leiden and prothrombin gene mutations did not differ significantly between groups , whereas, MTHFR C677T mutations and combined thrombophilias (Factor V Leiden and MTHFR C667T) were significantly increased in case group compared to controls group (Shalaby *et al*.,2016).

In (2000) spina and her colleges found that prevelance of factor VLeiden mutation in preeclampsia up to 26% (Spina *et al*.,2000).

In (2014) Dehkordi and his colleges study Association of Deficiency of Coagulation Factors (Prs, Prc, ATIII) and FVL Positivity with Preeclampsia and/or Eclampsia in Pregnant Women, and found that Statistical t-test indicated that the rate of FVL deficiency in pregnant patients with preeclampsia was significantly different from that in the control group (p=0.03) (Dehkordi *et al*.,2014).

In (2012) Khosravi and his colleges study Thrombophilic mutations and susceptibility to preeclampsia in Western Iran , and concluded that The frequency of heterozygous FVL mutation was 7.6% among all preeclamptic women (8.6% in mild and 5.7% in severe preeclamptic women) and 7.9% in controls (P[0.05). However, the prevalence of heterozygous FVL were 10.5 and 3.9% among severe preeclamptic women with early onset and late-onset preeclampsia, respectively (P 0.05) (Khosravi *et al*.,2012).

1.3 Rationale:-

Every year, over half a million women die worldwide of pregnancy-related complications. Ninety-nine per cent of these deaths occur in low- and middle-income countries,Complications of preeclampsia (PE) and eclampsia account for between 9–26% of these deaths .PE is also a major cause of fetal morbidity and mortality (Shamsi *et al*.,2013).

Evidence addressed the association of inherited thrombophilia with recurrent pregnancy loss, focusing on tests for three genetic variants; factor V Lieden , prothrombin G 20210A and methylene - tetrahydrofolate reductase (MTHFR).Associations between these heritable thrombophilia variants and other serious pregnancy complications as fetal growth restriction, placental abruption, preeclampsia, eclampsia, prematurity and intrauterine fetal death (Shalaby *et al*.,2016).

Many studies conducted in Sudan about factor V Leiden and its association with deep venous thromboembolism and recurrent miscarriage .There was no sufficient information about factor V Leiden among pregnant women with preeclampsia in Sudan.

1.4 Objectives:

1.4.1 General objective :-

To evaluate factor V Leiden mutation among pregnant women with preeclampsia syndrome.

1.4.2 Specific Objectives:-

-To detect factor V Leiden mutation among study volunteers using Polymerase Chain Reaction.

-To find possible correlation between mutation and other risk factor (age, hypertension , gravidity ,and other chronic disease).

Chapter two

Materials and methods

2.1 Study design:-

This was prospective case control and hospital based study.

2.2 Study area and Duration:-

The study was coducted in Omdurman Maternity Hospital in Khartoum state during the period from April to March 2019 .

2.3 Study Population :-

The study was conducted on pregnant women with preeclampsia as case group , and apparently healthy non-pregnant women as control group .

2.4 Inclusion Criteria :-

Case group : Pregnant women with preeclampsia.

Control group: Apparently healthy non- Pregnant women.

2.5 Exclusion criteria:-

Case group : Any pregnant women with other pregnancy complication gestational diabetes, preterm labor, fetal growth restrection , eclampsia , placental abruption, and intrauterine fetal death. While excluded any of those who reported with chronic disease (such as diabetes mellitus ,hypertension) or history of thrombosis and abortion among control group.

2.6 Sample size :-

This study included 50 case and 50 control individuals.

2.7 Sampling:-

Three ml of venous blood was collected from individuals under study and dispensed in EDTA container for PCR analysis .

2.8 Data Collection:-

Data was obtained by direct interviewing questionnaire. A questionnaire was designed to obtain information which helps in either including or excluding certain individuals in or from the study.

2.9 Principles and procedures:-

2.9.1 DNA Extraction :-

DNA will be extracted from whole blood using Guanidine hydrochloride protocol:

4ml of red cell lysis buffer (RCLB) will be added to each sample, and then samples will be centrifuged for 5min at 6000rpm ,the above step will be repeated 2 times until a clear pallet of white blood cell appear , supernatant will be discard then 2ml of white cell lysis buffer (WCLB),1ml of guanidine hydrochloride (57.2g dissolved in 100ml D.W),300ul of NH_4 acetate (57.81g dissolved in 100ml D.W) , and 10ul of protinase K will be added ,the sample will be incubated over night 37C° , After over night incubation the sample will be cooled at room temperature ,and then 2ml of pre-chilled chloroform will be added,after that sample will be centrifuged for 5min at 6000rpm , Upper layer will be collected to a new falcon tube , 10ml of cold absolute Ethanol will be added to collected samples , then kept at -20 C° overnight , After over night incubation the sample will be centrifuged for 10 min at 6000rpm, then the supernatant will be drained , pellet will be washed with 4ml of 70% ethanol. then will be centrifuged for 10min at 6000rpm ,and supernatant will be poured off and pellet will be allowed to dry Pellet will be dissolved in 100ul of ddH_2O and then will be incubated at 4 C° (Chomczynski and Sacchi,2006).

2.9.2 Principle of PCR:-

The PCR involves the primer mediated enzaymtic amplification of DNA.

PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered tempelate strand. Primer is needed because DNA polymerase can add a nucleotide only onto a preexisting 3-OH group to add the first nucleotide. DNA polymerase then elongate its 3 end by adding more nucleotides to generate an exended region of double stranded DNA (http://Laboratoryinfo.com , 2015).

2.9.3 Polymerase Chain Reaction :-

Each PCR-reactions was performed in a final volume of 20 uL containing 2.5 mM dNTPs , 2 uL target DNA , 1uL(10 pmol) of each primer (C, N or M) and 2.5U (5 U/ul) Taq polymerase.

Then the samples ran in a thermal cycler (eppendorf,mastercycler, vapoprotection).

ARMS-PCR was applied as a verifying test. In this method three primers were used: the wild-type primer is 5'GGACAAAATACCTGTATTCCTC3', the mutant primer is 5'GGACAAAATACCTGTATTCCTT3', and the common primer is 5'CTTTCAGGCAGGAACAACACC3' . The thermal cycling conditions consisting of 5 min denaturation at 95°C followed by 35 cycles of denaturation at 95 C° for 30 s, annealing at 56 C° for 45 s, extension at 72 C° for 45 s, then the final extension step at 72 C° for 10 min, kept at 4 C° until use.PCR reaction was run on 1 % agarose gel for 45 min at 120 V and stained with ethidium bromide (Dajani *et al*.,2012).

2.10 Ethical consideration :-

Participants were informed verbally in their simple language about the research, its benefits and method of sample collection ,then their approval taken, and ethical approval taken from ethical scientific committee and medical laboratory science of Sudan University of Science and Technology.

2.11 Statistical analysis :-

The Data collected , check and analyzed by using the statistical package for social sciences (SPSS) version 16 for interpretation of results significant difference will be determined using Chi square test less than 0.05,Mean , STD , Median and Frequency.

Chapter Three

3.Results

This study was carried out in Khartoum state at Omdurman Maternity hospital during period from April to March 2019.

Fifty volunteer preeclamptic women was enrolled in this study and matched group of apparently healthy control , with mean of age was 29.1 $\pm$ 6.1. In contrast the mean of age in control group was 29.5 $\pm$ 7.9 (Table 3.2).

According to age was classified into three groups (<20) , (20-35) , (>35). The most frequent one was 20-35 years , while the least one was < 20 years in case group. As like control group (Table 3.1).

In case group Regarding to the educational level , 6% of women had illiterate ,36% had higher education level. Healthy women were highest frequency compared with women have hypertension (80% , 20%) respectively, most of them (18%) use Nifedipine and 2% use Amilo5. According to gravidity high frequency was primy gravidity 32%, followed by secondy gravidity and grand multi gravity 28% and then the multi gravidity 12% (Table 3.1).

Eighty six of women not have thrombosis history but 14 % of them have history of thrombosis , regard to the abortion 40% have history of abortion and 60 % not have history , high frequency of abortion number is one time 22%, two time 14% and three time 4% (Table 3.1).

In control group regarding to the educational level , 4% of women had illiterate ,38% had higher education level . According to gravidity high frequency was primy gravidity 8%, followed by secondy gravidity and multi gravidity 38 % and then the grand multi gravity 16% (Table 3.1).

Table (3-1) Distribution of demographic data among study volunteers

Variables	Frequency / percent in case	Frequency / percent in control
Age Group		
Less than20	4 /50, 8%	6 /50, 12%
20-35	38 /50, 76%	31 /50, 62%
More than 35	8 /50, 16%	13 /50, 26%
Education level		
Primary	16 /50, 32%	13 /50, 26%
Secondary	13 /50, 26%	16 /50, 32%
College	18 /50, 36%	19 /50, 38%
Illiterate	3 /50, 6%	2 /50, 4%
Chronic disease		
Yes	10 /50, 20%	
No	40 /50, 80%	
Type of disease		
Hypertension	10 /50, 20%	
Type of drug		
Nifedipine	9 /50, 18%	
Amilo 5	1 /50, 2%	
Gravidity		
Primy	16 /50, 32%	4 /50, 8%
Secondy	14 /50, 28%	19 /50, 38%
Multi	6 /50, 12%	19 /50, 38%
Grand multi gravity	14 /50, 28%	8 /50, 16%
Histoty of thrombosis		
Yes	7 /50, 14%	
No	43 /50, 86%	
Abortion		
Yes	20 /50, 40%	
No	30 /50, 60%	
Number of abortion		
No history	30 /50, 60%	
One time	11 /50, 22%	
Twice	7 /50, 14%	
Third	2 /50, 4%	

Table (3-2) Mean ,Median and STD of Age and number of abortion in case

Variable	Mean± STD	Median
Age in case	29.1± 6.12	29
Number of abortion in case	0.61± 0.87	00
Age in control	29.5± 7.98	29

Table (3-3) Comparison in mean of age between case and control

Variable	Case	Control	*P.value*
Age	29.1 ± 6.1	29.5 ± 7.9	0.56

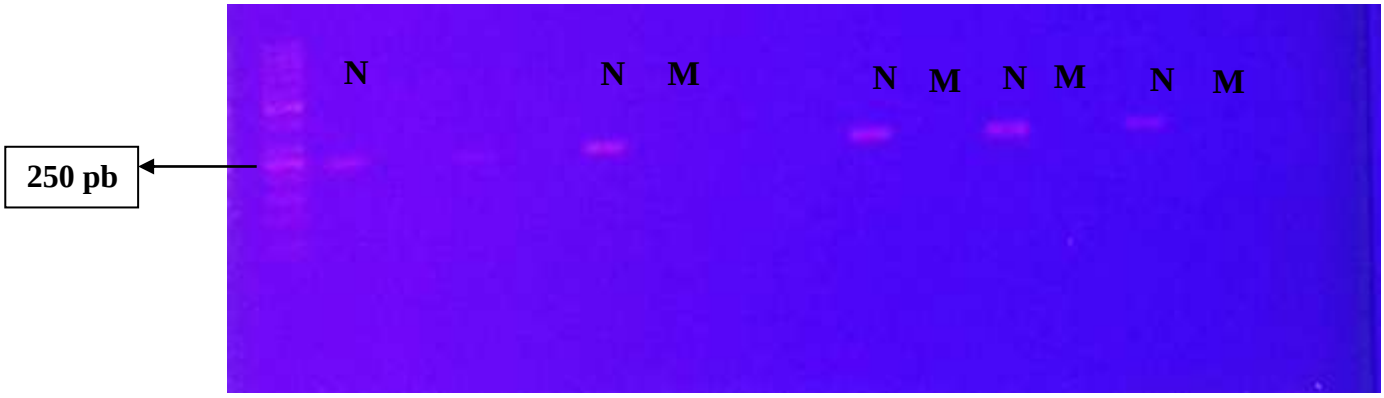

Figure 2 ARMS- PCR for detecting Factor V Leiden mutation in DNA isolated from whole blood samples of preeclamptic Sudanese women. Photograph shows ethidium bromide –stained 2% agarose gel , 50bp DNA ladder . 250 bp factor V PCR product.

N : normal ,M : mutant . the result show all sample are normal.

Chapter four

Discussion , Conclusion and Recommendations

4.1 Discussion:-

This is case control study conducted in Omdurman Maternity hospital during the period from April to March 2019.The study performed to find out subsequent effect of preeclampsia on factor V gene. It include 50 preeclamptic women and 50 age and sex matched group of apparently healthy control , age of participant range from 18-49 years.

The present study reflect that there is no significant relationship was observed between Factor V Leiden and preeclampsia .This is agreement with the study of (Salari *et al* .,2016) in the study the association between preeclampsia and defined polymorphisms in prothrombin and coagulation factor V genes which found that, there is no statistically significant difference.

And disagree with the study of karimi which found the incidence of factor V Leiden was typically higher in preeclamptic women than control group (Karimi *et al* .,2012).

The study show that age was not found as risk factor for preeclampsia and this is agree with study of Shamsi which found that the body mass index, maternal age, urinary tract infection, use of condoms prior to index pregnancy and sociodemographic factors were not associated with higher risk of having preeclampsia among Pakistani women (Shamsi *et al*.,2010).
In this present study no significantly different in terms of maternal age between two group *p.value* =0.56.This is agreement with the study of Karimi which found that there is no significantly different in age *p.value* = 0.9(Karimi *et al* .,2012) ,and disagree with the result of salari which show significant difference in the mean of age between case and control (Salari *et al* .,2016).

In our study the percent of cases have history of thrombosis was 14% ,86% not have history while in El baz study was 42% have history and 50% not have history(Elbaz *et al*.,2014).

In this study according to education level 6% illiterate , 32% have primary education , 26% have secondary education and 36% college education and in Bilano study he

found 2.9% , 4.3% , 4% , 4.1 % respectively, and he found 28% of cases have hypertension and we found 20% of cases have hypertension (Bilano *et al*.,2014).

In Dehkordi study show 23% of women with history of abortion while in our study show 40% of women have abortion history(Dehkordi et al.,2014).

Our result show that all gene was normal and not mutant ,this may be due to sample size , the mutation may be found in other site of exon that we work in it or may be presence on other exons

4.2 Conculusion:-

- Normal Factor V in preeclamptic women.

- There is no significant difference between factor V Leiden among case and control group.

- There in no correlation between factor V Leiden mutation and other risk factors such as age, gravidity, and chronic disease.

4.3 Recommendation :-

- Further study should be done by using large sample size and advanced techniques.

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Appendix I

Questionnaire

Sudan University of Science & Technology

College of Graduate Studies

Evaluation of Factor V Leiden in pregnant women with preeclampsia

تقيم وجود طفرة جينيه في عامل التجلط رقم 5 وسط النساء الحوامل المصابات بتسمم الحمل

Name :

year. 1/ Age

2/ Education level

3/ History of chronic disease ?

No Yes

If the answer is yes, specify ? and type of drug uses ?

4/ The number of pregnancies ?

5/ history of thrombosis in family?

Yes No

6/ History of abortion?

Yes No

If the answer is yes, number of abortion

Appendix II

Sudan University of Science & Technology

College of Graduate Studies

Evaluation of Factor V Leiden in pregnant women with preeclampsia

تقيم وجود طفرة جينيه في عامل التجلط رقم 5 وسط النساء الحوامل المصابات بتسمم الحمل

Consent Form

نموذج الموافقه

تسمم الحمل هو حاله طبيه يصاحب الحمل فيها ارتفاع ضغط الدم ، وظهور كميات ملحوظه من البروتين في البول . وقد اجريت االعديد من الدراسات لمعرفه عوامل الخطر للاصابه بهذه الحاله ، ووجد في بعض الدراسات ان للطفره الجينيه في عامل التجلط رقم 5 علاقه به.

وهذه الدراسه تهدف لتقيم وجود الطفره الجينيه في عامل التجلط رقم 5 وسط النساء المصابات بتسمم الحمل ونقوم بجمع المعلومات وعينات دم وريدي لعمل الفحوصات اللازمة ، لذا نرجو كريم فضلكم بالمشاركه في هذا البحث بالموافقه على اخذ المعلومات و العينات بغرض البحث واتعهد بأن استخدم كل النتائج في الأغراض البحثيه فقط مع الملاحظه على سريه الدراسه والنتائج .

اسم المتعاون في البحث :..... الامضاء:

.....

اسم الباحث :..... ت **:.....**

Appendix III

Laboratory Requirements

A. Reagents:

- DNA extraction:

solutions :

1- Red cell lysis buffer :

NH_4CL 8.3g

$KHCO_3$ 1.0g

EDTA 5% 1.8g

Compelet to 1000 ml D.W.

2- White cell lysis buffer :

Tris HCL 7.88g.

EDTA 7.44g

Sodium choloride 1.45g

SDS 1.0g

Dissolved in 500ml D.W.

3- Guanidine hypochloride 57.2g/100ml D.W.

4- NH_4acetate 57.81g/100ml D.W

5- proteinase K

6- Chloroform -20C

7- Absolute ethanol -20C.

- Master Mix

- Ladder

- Primers

- Agarose gel

B. Equipments:

- Sterile disposable plastic syrings.

- Cotton

- Ethanol tabs.

- Gloves

- Blood containers

- Falcon tube

- Eppendorf tube

- White tips

- Yellow tips

- Blue tips

- Automatic pipettes

- Pasteur pipettes

- Centrifuge

- Master cycler (eppendorf)

- Electrophoresis

- UV Light

- Sensitive balance

- Flask

Graduated cylinders-

Incubater -

Appendix IV

Master cycler (eppendorf)