Deanship of Graduate Studies
AL-Quds University

The Relationship between Factor V Leiden Mutation and Recurrent Abortion Among Palestinian Pregnant Women In The West Bank

U'la Daoud Khalil Abu Hilal

M.Sc.Thesis

Jerusalem-Palestine

1430/2009

The Relationship between Factor V Leiden Mutation and Recurrent Abortion Among Palestinian Pregnant Women In The West Bank

Prepared By:
U'la Daoud Khalil Abu Hilal

B.Sc: Major Biology-Minor Medical Technology
Al-Quds University-Jerusalem

Supervisor: Professor Hisham Darwish

A thesis Submitted in Partial fulfillment of requirements for the degree of Master of Biochemistry-Molecular Biology
Faculty of Medicine- Al-Quds University

1430/2009

Al-Quds University
Deanship of Graduate Studies
Biochemistry-Molecular Biology/Faculty of Medicine

Thesis Approval

The Relationship between Factor V Leiden Mutation and Recurrent Abortion Among Palestinian Pregnant Women In The West Bank

Prepared By: U'la Daoud Khalil Abu Hilal
Registration No: 20411415

Supervisor: Professor Hisham Darwish

Master thesis submitted and accepted, Date:

The names and signatures of the examining committee members are as follows:

1-Head of Committee:	Signature
Prof. Hisham Darwish	
2-Internal Examiner:	Signature
Dr. Sameer Barghouthi	
3-External Examiner	Signature
Dr. Ayman Hussein	

Jerusalem-Palestine

1430 /2009

Dedication

This work is dedicated to my great mother for being there for me whenever I need her, to my family; sisters and brothers for their love and motivation .To my husband's family for their love and support.

A special dedication to my dear husband Hasan for his love, support and endless patience. To my sons; Abdullah ,Mohammed Al Bashir, my sweet little baby Ayoub, and my lovely daughter Hibatallah for their understanding , love and patience .

To the memory of my father and my two beloved brothers Ali and Ayoub, may God mercy be upon them.

To my teachers; especially my supervisor Pro. Hisham Darwish for the great chance he offered me to be one of his students,
To all my friends who believed in me and support me no matter what.

Finally I would like to dedicate this work to the memory of Dr.Yaseen Jaber for the great efforts he did to the Palestinian society.

To these all I dedicate this work to represent my appreciation and grateful for all what they have done to me

U'la Daoud Khalil Abu Hilal

Declaration:

I Certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or part of the same) has not been submitted for a higher degree to any other university or institution.

Signed

U'la Daoud Khalil Abu Hilal

Date:

Acknowledgements

First of all I would like to thank My Great God for the faith, strength and patience he gave me through out my life.

My great and deepest thanks to Al Quds University, Faculty of Medicine represented by all the Professors and Doctors who taught us , and the Medical Research Centre for all the facilities, equipments and materials they provided us with during our study.

My deepest thanks and gratefulness to my supervisor Pro. Hisham Darwish for his kindness, patience, great experience, continuous encouragement and fruitful advice.

I would like to extend my deepest thanks to the following persons for their great efforts in helping us collecting data and samples from participants for our study:

Mrs. Nadia Mujahed and the whole stuff of AL Hiba Centre –Ramallah.
Dr Bahij al khayat and Lou′ie Handal from the Holy Family Hospital – Beit Lehem.
Dr Eman al A′nnany and her stuff from Palestinian Medical Relief society Clinic / Jericho.

Special and deepest thanks to Mr. Mujahed Deriah, Miss Renad Saleh and all my friends from Alquds University and Dr. Khaled Shelbayeh from An-Najah university for their support, help and encouragement.

Abstract

The Relationship between Factor V Leiden Mutation
and Recurrent Abortion Among Palestinian Pregnant Women
In The West Bank

The main objective of this study was to investigate the association between factor V Leiden mutation and Recurrent Abortion among Pregnant Women in the palestinian community in the West Bank. In order to focus the light on this major health problem and to figure out the best solutions to eliminate it from our palestinian community.

A questionnaire was developed and administered, 104 women were chosen, the case group included 54 women with no history of high blood pressure, and a history of recurrent pregnancy loss of unknown causes. The control group included 50 healthy women , who had 2 or more successful pregnancies, and none of them had a history of fetal loss or complicated pregnancy.

This research was carried out in the Medical Research Center at Al-Quds University, in Abu Dies (2007-2008), where Factor V Leiden mutation (FVL) was screened by PCR method using the ARMS test technique. Blood samples were collected from participants for DNA extraction in association with AL Hiba Center /Ramallah, Holy Family Hospital /Beithlehem and Palestinian Medical Relief society Clinic / Jericho.

The percentage of abortions among the participants in the case group was very high 68 % while all the pregnancies in the control group ended successfully. The distribution of factor V Leiden genotype among the case group was 27.8% (24.1% heterozygous alleles + 3.7 % homozygous alleles).While in the control group 22 % were heterozygous mutant ; which is statistically not significant (P value 0.324).
14% of the OCPs users among the case group were heterozygous for Factor V Leiden, while all the OCPs users among the control group were of normal genotype. The prevalence of IUFD among the participants was 11.1 % all normal for factor V genotype. There was no significance association between IUFD and Factor V Leiden mutation.

First trimester aborters were more prevalent (98.15%) than second trimester aborters (1.85%) ,the distribution of factor V Leiden mutation among the first trimester aborters (28.3 %) was higher than the second trimester aborters (0.0 %) mutant genotype.

The percentage of the primary aborters was 59.3 % whereas the secondary aborters 40.7 %. While the distribution of Factor V Leiden mutation among the secondary aborters (31.8% heterozygous alleles) was higher than those among the primary abortion (25 %= 6.30% homozygous alleles + 18.7 % heterozygous alleles).

These results indicate that statistically Factor V Leiden has no significant role in recurrent abortion. The high prevalence of Factor V Leiden among both the case and the control groups suggests that further large future studies on other DNA assays should be done for those women suffering from recurrent abortion including the genes encoding the natural anticoagulants antithrombin, protein C, and protein S, which results in a loss of anticoagulant function. Also the role of placental pathogenic mechanisms requires further evaluations.

More investigations and studies should be done taking into consideration the genotype of the women couples in order to predict fetus genotype.

Finally we recommend that we must focus our efforts on increasing the public awareness with regard to recurrent abortion and the importance of making the histopathology test for the abortuses in order to know the possible reason for abortion. The role of placental pathogenic mechanisms requires further evaluations. We should concentrate our efforts on the great role of the medical personnel among our populations in providing the necessary clinical care, including early diagnosis, medical consolations and therapy to improve the quality of lives of those people who suffered from it.

Content

List of Tables

List of Figures

Definitions :

ARMS: Amplification-refractory mutation system (ARMS)

Amino Acid: The basic building blocks for proteins in the body. Some Amino acids can be made by the body from other substances (nonessential) others are absorbed after ingestion of (essential) foods with protein (essential) .

Annexin V (human placental anticoagulant protein 1): Annexin V is an anionic phospholipid (PL)-binding protein, expressed by placental and vascular endothelium. It plays a thromboregulatory role at the vascular-blood interface by shielding anionic PL from forming a complex with coagulation proteins in the circulation.

Carrier: An individual who has a recessive, disease-causing gene mutation at a particular locus on one chromosome of a pair and a normal allele at that locus on the other chromosome; may also refer to an individual with a balanced chromosome rearrangement.

Chromosome 1 is the largest human non sex chromosome, which spans about 247 million nucleotide base pairs, and represents about 8% of the total DNA in human cells. It is currently believed to have 3,148 genes and was the last completed chromosome, sequenced two decades after the beginning of the Human Genome Project. The number of variations of nucleotides (SNP or single nucleotide polymorphism) is about 740,000.

Coagulation: The process by which blood clots.

Complete abortion:- the fetus, placenta, and other tissues are passed with bleeding.

Consanguinity: Genetic relatedness between individuals descended from at least one common ancestor.

Deep Venous Thrombosis (DVT): A clot that forms in the deep veins of the legs.

DNA: (Deoxy ribo Nucleic Acid) Substance from which the genetic code is made.

Eclampsia - a severe form of pregnancy-induced hypertension resulting in seizures.

Ectopic pregnancy: an abnormal pregnancy in which the fertilized egg implants outside of uterus. About 2 percent of all pregnancies develop outside the uterus.

Embryo - conceptus between time of fertilization to 10 weeks of gestation.

Enzymes: Biological catalysts that act to speed up chemical reactions.

Exon: Coding sequence of DNA present in mature messenger RNA.

Factor: The enzymes of the coagulation cascade are referred to as clotting factors.

Fetus: from 10 weeks of gestation till time of birth.

First trimester: within the first 12 gestational weeks.

Full term: refers to the end of 36 weeks (nine months) from the first day of the woman's last menstrual period — the end of gestation. If a woman gives birth earlier than this, it is classed premature birth.

Gene: The basic unit of heredity, consisting of a segment of DNA arranged in a linear manner along a chromosome. A gene codes for a specific proteins or part of functional protein leading to a particular characteristic or function.

Genetic code: Carried on chromosomes, made up of DNA. Humans have 46 chromosomes. Each chromosome contains many genes which encode various traits.

Gestational age - time from last menstrual period (LMP) up to present.

Gravidity (G) - number of times the woman has been pregnant

Heterozygote: An individual who has two different alleles at a particular locus, where one allele is usually normal and the other abnormal.

Homozygote: An individual who has two identical alleles at a particular locus, one on each chromosome of a pair.

Hemophilia: Term describing the condition in which patients have an abnormal tendency to bleed because they are unable to adequately form clots.

Homeostasis: The process by which blood flow is stopped. This is another word to describe the processes of clot formation.

Infarction: Lack of blood supply (and thus oxygen) to an organ or tissue resulting in tissue death.

Infant - time of birth to 1 year of age.

Intrauterine Fetal Death (IUFD): term for death of the fetus inside the uterus.

Intrauterine growth restriction (IUGR) - term for slowed growth of the fetus during pregnancy.

Ischemia: Lack of blood supply (and thus oxygen) to an organ or tissue .

locus: The physical site or location of a specific gene on a chromosome .

Leiden mutation : A glutamine-for-arginine (R506Q) substitution at factor V residue 506.

Miscarriage: an early pregnancy loss or spontaneous abortion.

Missed abortion - the embryo or fetus dies, but is not passed out of the uterus. Sometimes, dark brown spotting occurs, but there is no fetal heartbeat or growth.

Mutation: A genetic term. A mutation is a change in the genetic code from what is considered normal. Mutations can occur normally and not all mutations are harmful.

Myocardial Infarction (MI): This is another term for a heart attack. This occurs when a blood vessel (an artery) to the heart is blocked.

Parity (P) - number of pregnancies with a birth beyond 20 weeks GA or an infant weighing more than 500 g.

Placenta - an organ, shaped like a flat cake that only grows during pregnancy and provides a metabolic interchange between the fetus and mother. (The fetus takes in oxygen, food, and other substances and eliminates carbon dioxide and other wastes.)

Placental abruption - early detachment of the placenta from the uterus.

Placenta previa - placenta is attached close to or covering the cervix (opening into the uterus).

Platelets (Plt): Cells in the blood which serve to plug holes in the blood vessel walls and assist in forming a clot.

Polymerase Chain Reaction (PCR**):** A procedure that produces millions of copies of a short segment of DNA through repeated cycles of: 1) Denaturation, 2) Annealing, and 3) Elongation.

Polymorphism: A genetic term. Polymorphisms are changes in the genetic code (like mutations) that occur commonly enough in the population such that they are considered a variation on normal. These may be harmful, helpful or neither to the persons who have a polymorphism.

Postpartum: after delivery.

Point mutation: An alteration in DNA sequence caused by a single nucleotide base change, insertion, or deletion .

Preclampsia - a condition characterized by pregnancy-induced high blood pressure, protein in the urine, and swelling (edema) due to fluid retention.

Premature - describes a baby born before 37 weeks of pregnancy.

Prenatal diagnosis: (synonym: prenatal testing) Testing performed during pregnancy to determine if a fetus is affected with a particular disorder.

Preterm - occurring before 37 weeks of pregnancy.

Preterm infant - delivered between 24-37 weeks.

Previable infant - delivered prior to 24 weeks.

Primary abortion: the result of the first pregnancy is abortion (no children before).

Protein: Proteins are essential molecules in the body made up of many amino acids strung together. DNA encodes the proteins and the cells can then turn the DNA into RNA and ultimately into proteins. Clotting factors are one of many types of proteins.

Recurrent Pregnancy Loss (RPL): Three or more spontaneous fetal losses before the 20 week of pregnancy

Restriction site: A sequence of DNA that is recognized by an Endonuclease (a protein that cuts DNA) as a site at which the DNA is to be cut.

Secondary abortion: Abortion occur subsequent to having children; the result of the first pregnancy either normal or cesarean delivery.

Second trimester – 12 to22 weeks of gestation.

Stillbirth is a common term for death of a baby while still in the uterus. It is also called intrauterine fetal death or demise.

Sub endothelium: contains numerous proteins and other molecules which, when exposed to the blood stream, serve to attract platelets and encourage clot formation.

Term infant - delivered between 37-42 weeks.

Third trimester - 22 gestational week to delivery.

Thrombus: A blood clot.

Thrombophilia: Another term describing hypercoagulable states. These conditions confer an increased risk of forming clots.

Viability - minimum age for fetus survival, (. third trimester).

Zygote - from fertilization until second cell division

List of abbreviations :

1st trimester	First trimester
2nd trimester	Second trimester
3rd trimester	Third trimester
APC	Activated protein C
ARMS	Amplification refractory mutation system
DVT	Deep vein thrombosis
FVL	Factor V Leiden mutation
HRT	Hormone replacement therapy
IUFD	Intrauterine Fetal Death
IUGR	intrauterine growth retardation
IV	Intravenous
OCPs	Oral contraceptive pills
PCR	Polymerase chain reaction
PE	Pulmonary embolus
PL	phospholipid
R5o6Q	A glutamine-for-arginine (R506Q) substitution at factor V residue 506
RPL	Recurrent pregnancy loss
TAE	Tris-acetate-EDTA
TE	Tris- EDTA
VTE	venous thromboembolism
w/v	Weight/ volume

Chapter One
Introduction

Thrombophilia

Thrombophilia: defined as a predisposition to thrombosis. It is a multifactorial disorder caused by inherited and acquired defects, such as mutations in the genes encoding natural anticoagulants including antithrombin, protein C, and protein S, or clotting factors prothrombin and factor V **(**Walker. 2000). Acquired conditions include surgery, long distance immobilization, pregnancy, antiphospholipid syndrome, obesity, and use of oral contraceptive pills (OCPs) (Bertina et al .1994).

In recent years, a number of studies concerning the relationship between Recurrent Pregnancy Loss (RPL) and thrombophilias have been carried out in many countries around the world. Some studies reached conflicting conclusions about the role of Factor V Leiden and Recurrent Pregnancy Loss .This may be due to the fact that the incidence of Factor V Leiden Mutation in different populations is affected by racial and ethnic differences in the gene frequency *(*Burchard et al. 2003) .Factor V Leiden(a genetic variant) is found in about 5 % of caucasians, while, this variant is rarely found in East Asians and Africans (prevalence ≤1 %) (Ridker et al .1997). Rees et al found that the highest prevalence of the factor V Leiden mutation was found in Europeans (4.4%); especially among Greeks (7%) (Rees et al .1995).Among the population from Asia Minor the prevalence was (0.6%) while it was absent in Africa (Cleary- Goldman et al .2003)**.**

According to the National Human Genome Research Institute (www.http:// genome.gov) The use of hormones, such as oral contraceptive pills (OCPs) and hormone replacement therapy (HRT), including estrogen and estrogen-like drugs taken after menopause, increases the risk of developing Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE) .(www.http:// genome.gov) .Healthy women taking OCPs have a 3-4 increased risk of developing a DVT or PE compared with women who do not take OCPs. Women with factor V Leiden (FVL)who take OCPs have about a 35-fold increased risk of developing a DVT or PE compared with women without factor V Leiden and those who do not take OCPs. Women with thrombophilic defects are at increased risk, not only for pregnancy associated thromboembolism, but also for other vascular complications of pregnancy, including pre-eclampsia and fetal loss **(**Walker .2000).

1.1 Pregnancy

Normal pregnancy is associated with major changes in all aspects of haemostasis ;increased concentrations of most clotting factors, decreased concentrations of some of the natural anticoagulants, and reduced fibrinolytic activity (Christopher et al .1998).Consequently, as pregnancy progresses and during the puerperium the overall balance is shifted towards apparent hypercoagulability (Clark et al .1998).

Hypercoagulability appears to be an important feature in the pathogenesis of many of the complications of pregnancy including venous thromboembolism (VTE), pre-eclampsia (PE), intrauterine growth retardation (IUGR) , and fetal loss **(**Walker .2000).

Pregnant women ha**v**e 2–5-fold higher risk for **v**enous thromboembolism compared to non-pregnant women (Grandone et a1 .1997) , (Price and Ridker . 1997).The F**V**L **v**ariant allele increases the risk for venous thromboembolism during pregnancy 8–10 times, compared to non-pregnant women with the wild-type genotype (Price and Ridker .1997).

Recurrent pregnancy loss (RPL), classically defined as 3 or more spontaneous fetal losses before the 20^{th} week of pregnancy , is a health problem affecting 0.5 %-1.0 % of pregnant women (Cooper .1994) .RPL may be compounded by its significance as a symptom of a serious medical condition with lifelong implications. Miscarriage is a painful and, unfortunately, common event, occurring in about 20 % of all pregnancies, fewer than 50 % of these cases have definitive causes (Rees et al .1996).

Both inherited and acquired risk factors may be responsible ,these include parental chromosomal abnormalities (2%-3%), uterine abnormalities (10 % -15 %),hormonal imbalance (25 %) , and the antiphospholipid antibody syndrome (5 %-10 %) (Gynecologist journal no24,2004)**.**Thrombophilias such as Factor V Leiden mutation may be responsible for a large portion (40-60 %) of unexplained multiple miscarriages (Brenner et al.1997).

1.2 Pregnancy and Maternal Fetal Circulation

Once pregnancy is established, survival of the fetus depends on placental development. By the 17^{th} day after fertilization, the fetal blood vessels are functional, and placental circulation is established (Cleary – Goldman et al 2003). The human placenta is characterized by a dual arterial blood supply; maternal (in the intervillous space) and fetal

(in the blood vessels of the cord and villi) (Benirschke. 1998). The fetomaternal circulatory system may be compromised by disturbances of haemostasis ; a delicate balance between anticoagulant and procoagulant factors is needed to maintain this system (Cleary – Goldman et al.2003).Teleologically, it makes sense that the placenta must prevent hemorrhage so it is prone to infarction and fibrin deposition (Roberts. 2002) .

Annexin V produced by villous trophoblasts is a potent anticoagulant that has a key role in preventing intervillous thrombosis and maintaining the fluidity of the placental circulation, annexin V inhibits coagulation by binding to phospholipids, the concentration of maternal blood levels of factor VIII, fibrinogen, and other procoagulant proteins increases considerably during pregnancy in order to prevent placental hemorrhage (Roberts et al. 2002).

Heritable thrombophilia affects the dual function of the placenta, accordingly, risks increased for eclampsia, abruptio placenta, fetal growth restriction, and stillbirth (Kupferminc et al. 1999). Another study done by Roberts & coworkers has been hypothesized that a hypercoagulable state causes placental microthrombosis which can lead to placental infarctions, reduced placental blood flow, and possible fetal death (Roberts et al. 2002). The majority of studies on thrombosis and RPL focused on the maternal rather than fetal circulatory system (Cleary – Goldman et al .2003).

One study suggested that thrombosis can occur in either maternal or fetal circulation, a 2-fold increase in factor V Leiden carrier abortuses frequency (8.6%) compared with (4.2%) in unselected pregnant controls was found (Dizon-Townson et al 1997). They also found a 10-fold increase in the fetal carrier frequency in placentas with more than 10% placental infarction. In fact the frequency of the factor V Leiden mutation in miscarried fetuses has been found to be more than twice that in the general population (Dizon-Townson et al 1997) .The fetus can inherit the mutation from either maternal or paternal gene pool, thus it appears that the carrier status of the fetus, rather than that of the mother, may be the main consideration in fetal loss (Cleary – Goldman et al 2003).In a recent study done by Toth & coworkers they found that abortions in the embryonic phase of fetal development were associated with a significantly higher incidence of maternal heterozygosity for FVL (Toth et al .2009).

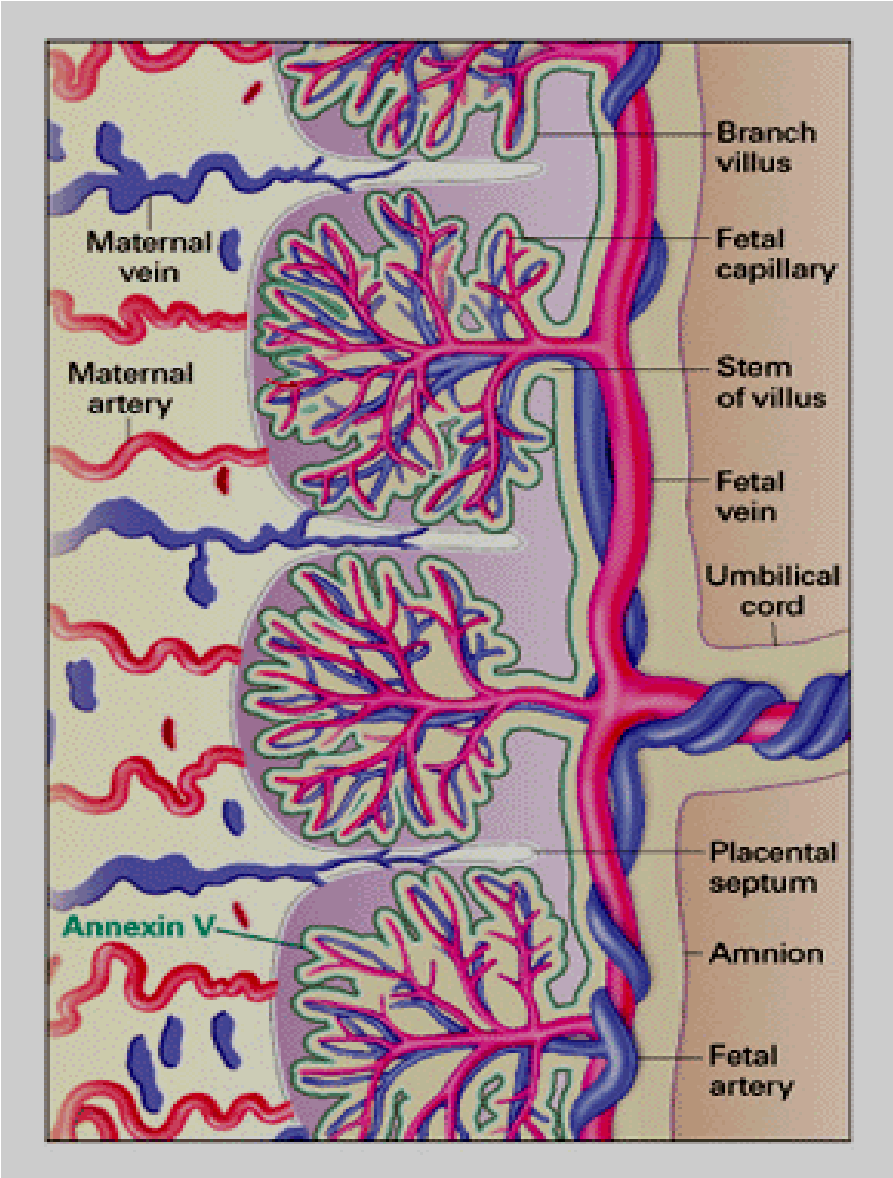


Figure 1.1 The Dual Circulation of Placenta.

Figure 1.1 the anatomy of the placenta. The fetal circulation is shown on the right-hand side, the maternal circulation on the left-hand side, and the placenta in the middle. The fetal circulation flows from the umbilical artery through the fetal stem and branch villi to villous capillaries. The villi, covered by trophoblasts (green) that express annexin V, are bathed in blood from the maternal space (lavender). The maternal space is filled from maternal decidual arteries and drains through maternal decidual veins. Maternal blood contains relatively high amounts of procoagulant proteins, whereas annexin V on the tips of the villi is a potent anticoagulant (Roberts et al .2002).

1.3 Recurrent Abortion

Spontaneous abortion (SAB), or miscarriage, is the term used for a pregnancy that ends on its own, within the first 20 weeks of gestation. Most miscarriages occur during the first 13 weeks of pregnancy. There are different types of miscarriage, different treatments for each, and different statistics for what a woman chances are of having one**.** The most common of these are:

- **A blighted ovum** : A blighted ovum occurs happens when a fertilized egg attaches itself to the uterine wall, but the embryo does not develop. Cells develop to form the pregnancy sac, but not the embryo itself. A blighted ovum usually occurs within the first trimester before a woman knows she is pregnant.

-**Missed Abortion:** the embryo or fetus dies, but is not passed out of the uterus. Sometimes, dark brown spotting occurs, but there is no fetal heartbeat or growth.

– **Ectopic:** an abnormal pregnancy in which the fertilized egg implants outside of the uterus, about 2 percent of all pregnancies develop outside the uterus.

- Still Birth : is a common term for death of a baby while still in the uterus > 20 gestational week and before delivery . It is also called intrauterine fetal death (IUFD) (http://www.pregnancy online).

1.4 Haemostasis (Coagulation and anticoagulation systems)

Haemostasis is the body's normal physiological response for the prevention and stopping of bleeding/hemorrhage. It results in the blocking of any vascular cut; it ensures blood fluidity and blood vessel integrity. Abnormalities in haemostasis can result in bleeding (hemorrhage) or blood clots (thrombosis) (http://stago.com).

Haemostasis consists of primary haemostasis with local vascular contraction (to reduce blood flow to the injury site)& platelet plugs formation , secondary haemostasis which involves clotting of the plasma that result from interaction between numerous factors and inhibitors of the clotting system, and finally fibrinolysis which is the process of removing the clot once blood vessel integrity has been restored (http://stago.com).

1.4.1 Coagulation system:

The intrinsic(contact activation pathway) and the extrinsic pathway consist of a series of reactions, in which a zymogene (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active enzymes that catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. The coagulation factors are generally serine proteases (enzymes) with some exceptions; for example, factor VIII and factorV are glycoproteins and Factor XIII is a transglutaminase. Serine proteases act by cleaving other proteins at specific sites. The coagulation factors circulate as inactive zymogens (Marz et al. 2000).The extrinsic pathway and the intrinsic pathway are activated as explained below:

The extrinsic pathway (Tissue factor pathway)

Following damage to the blood vessel, injuries of the endothelium and the exposure of the sub endothelial tissue to the blood stream tissue factor (TF, an integral membrane glycoprotein in the adventitia) is released, it comes into contact and forms a complex with factorVII. In the presence of calcium, factorVII binds to the extra cellular domain of the TF and is activated to factorVIIa (factorVIIa circulates in a higher amount than any other activated coagulation factor) this complex (TF-factorVIIa) in turn activates factor IX and factorX. FactorVII is itself activated by thrombin, as well as factorXIa, plasmin, factor XII and factor Xa. The activation of factor Xa by TF-factorVIIa is almost immediately

inhibited by tissue factor pathway inhibitor (TFPI). With the formation of factor Xa, the intrinsic and the extrinsic pathway converge. Together with its co-factor (factor Va), calcium and phospholipids, factor Xa builds up the prothrombinase complex which activates prothrombin to thrombin (Marz et al. 2000). Thrombin then by limited proteolysis converts soluble fibrinogen into insoluble fibrin. It also activates other components of the coagulation cascade, including factorV and factorVII (which activates factor XI which in turn activates factor IX), and activates and releases factor VIII from being bound to von willibrand factor (vWF). Factor VIIIa is the co-factor of factor IXa and together they form the "tenase" complex which proteolytically converts factorX to factor Xa and so the cycle continues. As shown later in figure 1.2 Coagulation cascade

The intrinsic pathway (Contact Activation pathway)

The formation of the primary complex on collagen by high molecular weight kininogen (HMWK), prekallikrein and factor XII (Hageman factor) . Prekallikrein is converted to kallikrein and factor XII becomes factor XIIa. factor XIIa converts factor XI into factor XIa. Factor XIa activates factor IX, which with its co-factor (factor VIIIa) form the tenase complex which activates factor X to factor Xa. Under physiological conditions factor IX is a better substrate for the TF- factor VIIa complex than factor X. This directly couples the intrinsic to the extrinsic pathway and explains why bleeding tendency is much more sever in factor IX deficiency than in deficiencies of other factors of the extrinsic pathway (Marz et al. 2000).

Thrombin has a large array of functions. Its primary role is the conversion of fibrinogen to fibrin, the building block of a haemostatic plug. In addition, it activates Factors VIII and factor V and the inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers. Following activation by the contact factor or tissue factor pathways the coagulation cascade is maintained in a prothrombotic

. state by the continued activation of factor VIII and factor IX to form the tenase complex, until it is down regulated by the anticoagulant pathways (Marz et al. 2000). As shown below in figure 1.5 Coagulation cascade

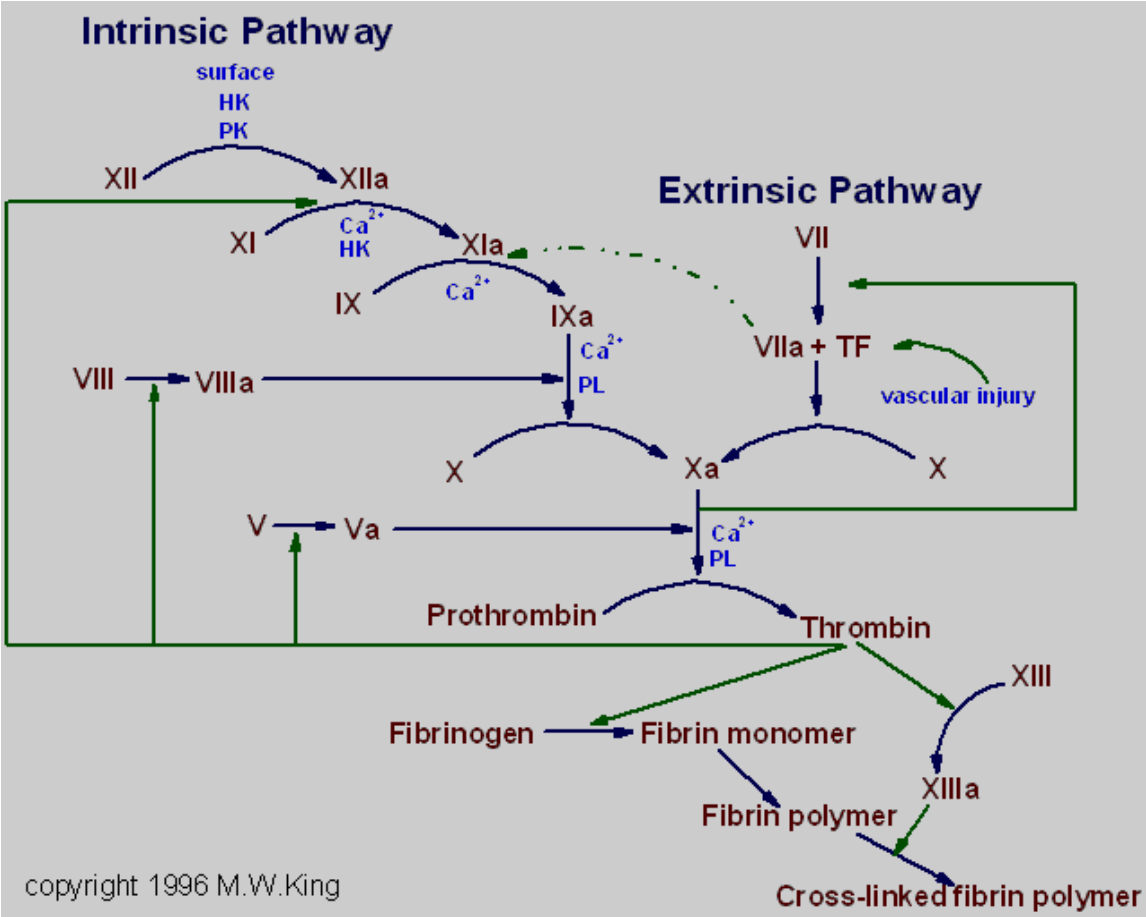


Figure 1.2 Coagulation cascade
(http://web.indstate.edu/thcme/mwking/blood-coagulation.htm#control)

Figure 1.2 : The coagulation cascades

The intrinsic cascade is initiated when contact is made between blood and exposed negatively charged surfaces. The extrinsic pathway is initiated upon vascular injury which leads to exposure of tissue factor (TF), a sub endothelial cell-surface glycoprotein that binds phospholipid. The green dotted arrow represents a point of cross-over between the extrinsic and intrinsic pathways. The two pathways converge at the activation of factor X to Xa. Factor Xa has a role in the further activation of factor VII to VIIa as depicted by the green arrow. Active factor Xa hydrolyzes and activates prothrombin to thrombin. Thrombin can then activate factors XI, VIII and V furthering the cascade. Ultimately the role of thrombin is to convert fibrinogen to fibrin and to activate factor XIII to XIIIa. Factor XIIIa (also termed transglutaminase) cross-links fibrin polymers solidifying the clot. HK = high molecular weight kininogen. PK = prekallikrein. PL = phospholipid.

1.4.2 Anticoagulation system (Protein C (PC)anticoagulant pathway):

Protein C (Figure 1.6) is a major physiological anticoagulant. It is a vitamin K-dependent serine protease zymogene, mainly synthesized in the liver. It is proteolytically activated on the surface of the endothelial cells by thrombomodulin bound thrombin (APC).APC provides physiologic antithrombotic activity and exhibits both anti-inflammatory and anti-apoptotic activities. Activated protein C (APC), in a complex with protein S, inactivates procoagulant factors Va and VIIIa by proteolytic cleavage at specific arginine residues. This serves to control coagulation and limit the extent of thrombus formation. The specific cleavage sites on factorVa occur at arginine residues 506,306,679 while those on factorVIIIa are at arginine residues 562,336,740. APC requires calcium, phospholipids and Protein S (PS) as cofactors.

Normally factorVa is inactivated by an initial cleavage of the peptide bond on the carboxyl side of arginine 506 followed by a second cleavage at arginine 306. The mutant factorV is inactivated by cleavage at arginine 306, but this cleavage is ten fold slower without prior cleavage at position 506 which is prevented by the factorV Leiden mutation. Thus the factorV Leiden mutation leads to the phenomenon of resistance to the anticoagulant activity of APC.

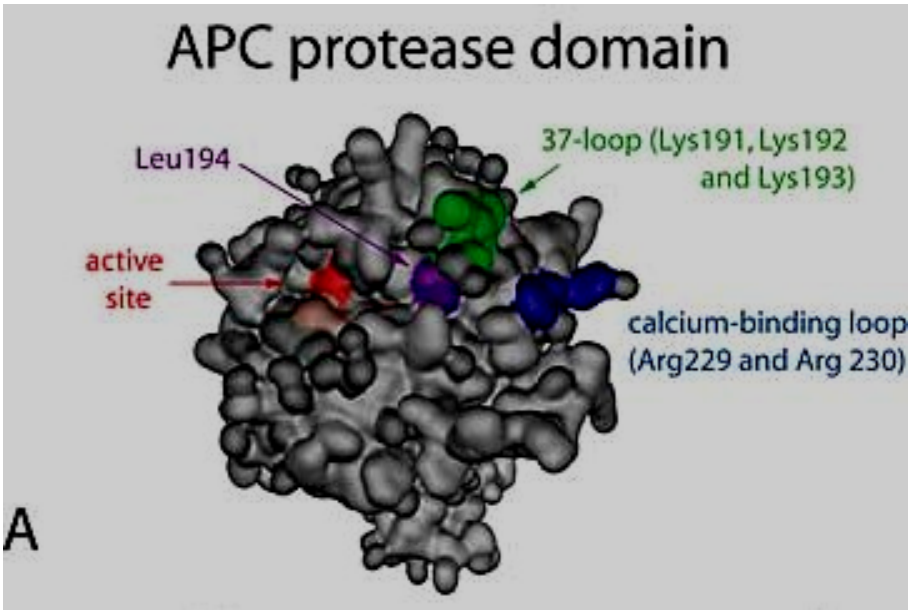


Figure 1.3 Protein C (inactivator of coagulation factors Va and VIIIa) (Mosnier et al.2006)

The Protein C (PC) anticoagulant pathway represents an important regulator of the blood coagulation as shown in Figure 1.4

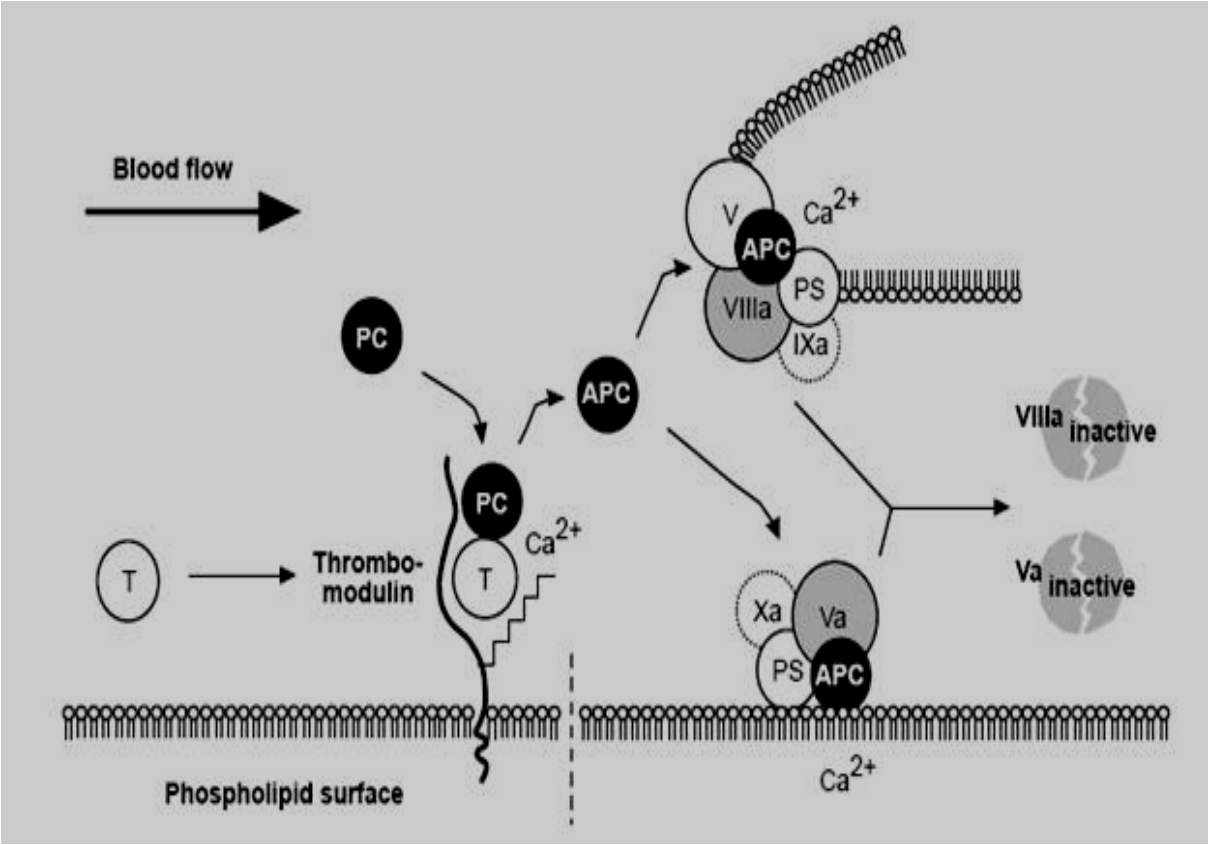


Figure1.4 The Protein C anticoagulant pathway. (Marz et al. 2000)

Thrombin escaping from a site of vascular injury binds to its receptor thrombomodulin (TM) on the intact cell surface; the endothelial PC receptor (EPCR). Thrombin loses its procoagulant properties after binding to(EPCR) and becomes a potent activator of protein C. Activated protein C(APC), together with its cofactor, protein S, inactivates factors Va and VIII a to provide negative feedback to the generation of thrombin. (APC) functions as a circulating anticoagulant, which specifically degrades and inactivates the phospholipid-bound factors Va and VIIIa. This effectively down-regulates the coagulation cascade and limits clot formation to sites of vascular injury. This result in down regulation of the tenase complex (factor VIIIa+ factor IX a) and "prothrombinase" complexes (Marz et al. 2000)

The endothelial PC receptor (EPCR) is a transmembrane protein belonging to the major histocompatibility class 1 family of molecules, it is a recently identified regulatory component of the PC anticoagulant pathway it has two functions :
1- To amplify the conversion of PC to APC by thrombin and thrombomodulin.
2- To inhibit the anticoagulant activity of APC by shifting the specifity of APC away from FVa and FVIIa (Marz et al. 2000).

In factorVa three peptide bonds located at Arg-306, Arg-506, and Arg679 are sensitive to proteolysis by APC. The three cleavage reactions have distinct kinetic properties; and dependence on phospholipid, differently stimulated by protein S, and yield FVa-inactivation intermediates with different FVa activities. The cleavage at Arg-506, which is kinetically favored, is not dependent on protein S and yields a (FVa) inactivation intermediate with decreased FXa-cofactor activity. The APC-mediated cleavage at Arg-306 is stimulated by protein S and results in almost complete inactivation of FVa .This loss of factorVa activity is partly due to reduced affinity for FXa, but the Arg-306 cleavage also leads to dissociation of the A2 domain which is crucial for full loss of function. The cleavage at Arg-506 may occur in the absence of phospholipid, whereas the cleavage at Arg-306 is more dependent on the presence of phospholipid (Yong-Hui Sun et al.2004).

1.4.3 Activated protein C resistance:

The concept of resistance to APC was first introduced in 1993 by Dahlbäck and associates (Dahlbäck et al .1993). Koster and coworkers in 1993 reported that this phenotype was a risk factor for developing a first Deep Vein thrombosis (DVT). In 1994, Bertina et al, reported that >80% of cases with APC resistance were carriers of the same mutation in the gene of factor V; a G $\rightarrow$A transition in position 1691, in Exon 10, that predicts the replacement of Arg 506 by Gln in factor V molecule (factor V Leiden) (Bertina et al 1994). This mutation slows the inactivation of Factor Va by APC causing a hypercoagulable state. The presence of Factor V (Leiden) is the most common cause of inherited thrombophilia, accounting for 20% to 50% of cases (Bertina et al 1994).

1.5 Factor V (Gene, Protein & Mutation)

The gene for factor V is located on the long (q) arm of chromosome 1 at position 23. It is genomically related to the family of multicopper oxidases, and is homologous to coagulation factor VIII. The gene spans 70 kb, consists of 25 exons, and the resulting protein has a relative molecular mass of approximately 330000 (330 k Da).

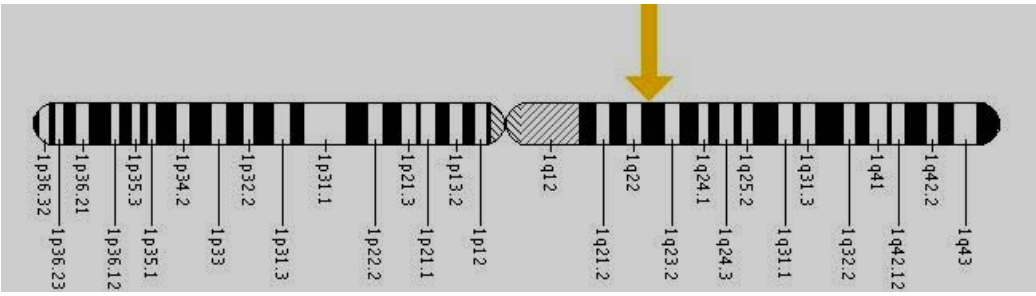


Figure 1.5 Cytogenetic Location of Factor V is: 1q23.

(ghr.nlm.nih.gov/dynamicImages/chromomap/f5.jpeg)

Factor V gene present in three main forms; normal homozygous gene with both alleles normal, heterozygous (one allele normal and the other mutated) and homozygous mutant (both alleles mutated). Mutations in this gene may results in different disorders in the blood clotting system; some mutations in the FV gene prevent the production of a functional factor V protein or decrease the amount of the protein in the bloodstream. When present in two copies of the F5 gene, these mutations lead to a rare condition called factor V deficiency or Para hemophilia. A reduced amount of functional factor V prevents blood from clotting normally; causing episodes of abnormal bleeding that can range from mild to severe.

1.5.1 Factor V protein:

Factor V official symbol is F5, also known by other names such as: coagulation factor V, proaccelerin or labile factor, activated protein c cofactor, coagulation factor V, Jin jiang A2 domain. Factor V circulates in plasma as a single-chain molecule with a plasma half-life of about 12 hours. It is made chiefly by cells in the liver as a single-chain precursor glycoprotein of 2,224 amino acids (aa), consisting of a 28-aa N-terminal signal peptide, followed by the 2,196-aa mature protein ≈330 k Da undergoing extensive posttranslational modifications before being secreted into the blood (Wilson et al .1984).In contrast to most other coagulation factors, it is not enzymatically active but functions as a cofactor (Gerry et al. 2002).

Factor V plasma concentration is ≈20 nmol/L (≈0.007 g/L), it has two forms ; either circulating in free form in plasma or present in the α-granules of platelets; this form accounts for ≈25% of the total FV content in human blood (Tracy et al. 1982). During coagulation, platelet FV is secreted as a result of platelet activation, yet it is unclear whether the presence of FV in platelets is the result of the uptake of exogenous FV from the circulation via endocytotic processes by megakaryocytes or whether these cells themselves can account for the FV production (Gerry et al. 2002).

1.5.2 Factor V Function:

Factor V (FV) is a central regulator of homeostasis, serving both as a critical cofactor for the prothrombinase activity of factor Xa and the target for proteolytic inactivation by the anticoagulant, activated protein C (APC).Factor V is able to bind to activated platelets and is activated by thrombin. On activation, factor V is spliced in two chains (heavy and light chain with molecular masses of 110000 and 73000, respectively) noncovalently bound to each other by calcium. Factor V is active as a cofactor of the thrombinase complex (Hajjar.1994).

Factor V has procoagulant and anticoagulant action as shown next in figure 1.2

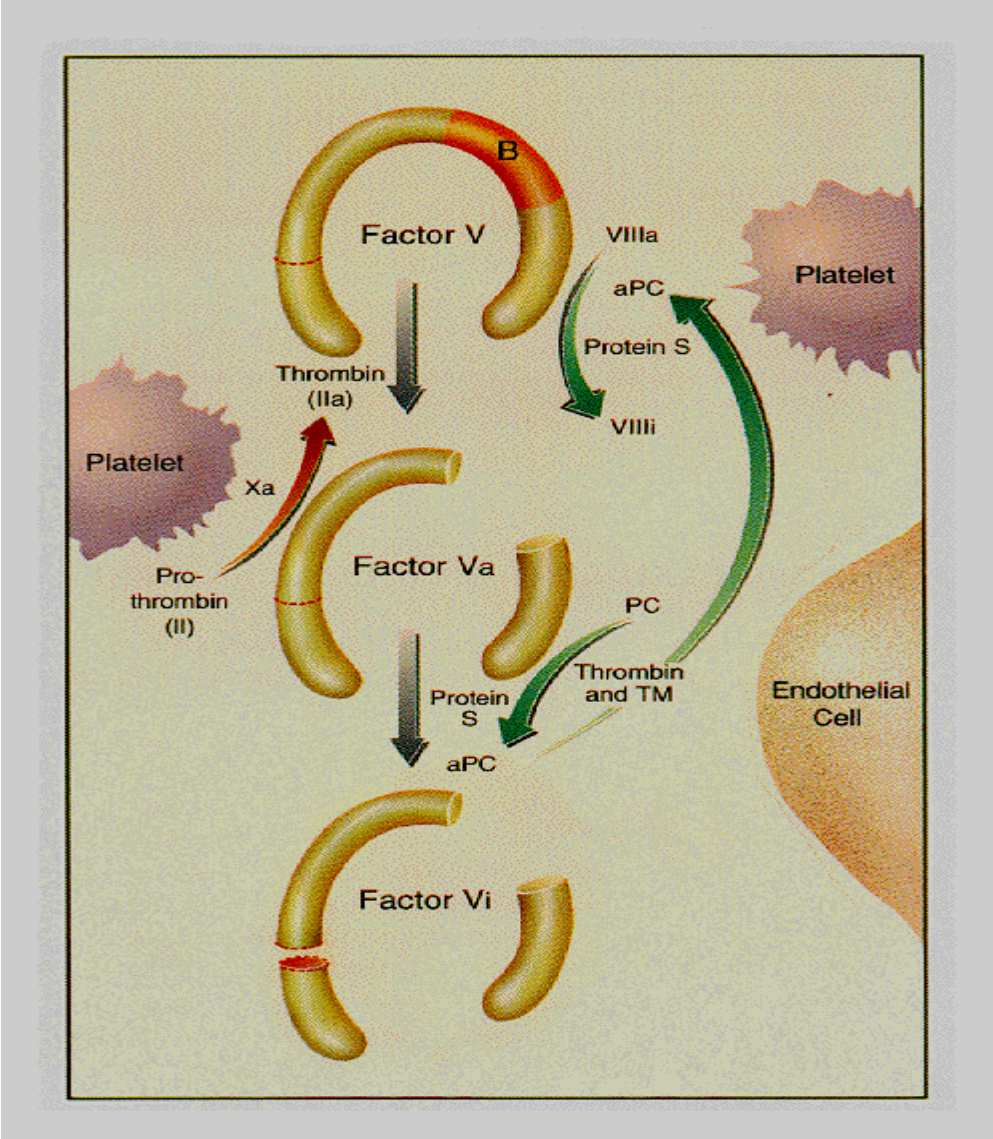


Figure 1.6 Some Procoagulant and Anticoagulant Actions of Factor V (Hajjar.1994).

Procoagulant and Anticoagulant Actions of Factor V

Before activation, factor V is a single-chain polypeptide. On the surface of an activated platelet Factor V can serve (with protein S) as a cofactor in the inactivation of factor VIIIa by activated protein C (APC) (Dahlbäck et al.1994).Factor V is activated to become a two-chain polypeptide, factor Va (middle), when its B domain is excised by thrombin (factor IIa). On the platelet surface, factor Va enhances the activation of prothrombin (factor II) by factor Xa. When thrombin binds to its receptor, thrombomodulin (TM), on the surface of endothelial cells, it activates protein C (PC). Factor Va augments this action. The APC inactivates factor Va (which becomes factor Vi) in the presence of protein S by cleaving a single peptide bond, Arg506-Gly507 (dotted line) in factor Va. Procoagulant and anticoagulant actions are indicated by red and green arrows, respectively (Hajjar.1994).

1.5.3 Factor V Leiden:

A variant of human factor V that causes a hypercoagulability disorder (Factor V Leiden Thrombophilia) named after the city Leiden (The Netherlands), where it was first identified by Prof R. Bertina and associates (Bertina et al. 1994) .

Factor V Leiden is the most common inherited form of thrombophilia, factor V Leiden (factor V R506Q, G1691A) is the most common genetic prothrombotic defect, with an overall prevalence of carriers among Caucasians around 5%, It is found in 20% of all patients with venous thrombosis, and in up to 50% of patients with thrombophilia (Rosendal .2005)**.**

Factor V Leiden leads to resistance to APC- as the result of the mutation of one of the cleavage sites where APC inactivates factor V. Because inactivation of the procoagulant mutant factor V occurs less efficiently, 'factor Va persistence' leads to an increased risk of thrombosis. Factor V Leiden mutation (Arg506Gln) is present in 4 to 10% of people of Caucasian origin (Bertina et al. 1994,Rees. 1996). The factor V Leiden mutation induces a hypercoagulable state which increases the risk of venous thrombosis seven-fold among heterozygous carriers and about eighty-fold among homozygous carriers compared to non-carriers (Rosendal et al. 1995). It has been suggested that factor V Leiden mutation may be associated with negative outcomes of reproduction such as recurrent abortion, pre-

eclampsia, prematurity and small for- gestational-age neonates (Grandone et al .1997, Ridker et al. 1998, Brenner et al .1999).

Mutation of the gene encoding factor V(The APC resistance phenotype) is associated with a guanine-to-adenine substitution at nucleotide 1691 of the factor V gene. This mutation produces a glutamine-for-arginine (R506Q) substitution at factor V residue 506 **(**factor V Leiden**)** as shown in the Figure1.7**.** Since this amino acid is normally the cleavage site for APC, the mutation prevents efficient inactivation of factor V (Hillarp et al.1996).

Factor V Leiden (R506Q)		
505	506	507
Arg	**Arg**	Gly
AGG	C **G** A	GGA
	↓	
AGG	C **A** A	GGA
Arg	**Gln**	Gly

Figure 1.7 The G to A point mutation results in the Arginine at the protein C cleavage site being replaced by Glutamine.

When factor V remains active, it facilitates overproduction of thrombin leading to excess fibrin generation and excess clotting .The excessive clotting that occurs in this disorder is almost always restricted to the veins, where the clotting may cause a deep vein thrombosis (DVT). If the venous clots break off, these clots can travel through the heart to the lung, where they block a pulmonary blood vessel and cause a pulmonary embolism.

Several mutations at the Arg306 residue in factor V have been described in patients with a history of thrombosis. These include replacement of Arg306 with threonine (factor V Cambridge) (Williamson et al.1998) or with glycine (Chan et al .1998)**.** Occasionally, patients have been described who have heterozygous APC resistance due to the factor V Leiden mutation and type I factor V deficiency (Guasch et al. 1997) .The plasma of these individuals manifests severe APC resistance in activated partial thromboplastin time assays, similar to that seen in patients with homozygous factor V Leiden. These patients

appear to be more thrombosis prone than their heterozygous relatives with factor V Leiden alone, suggesting that the clinical phenotype is similar to patients who are homozygous for factor V Leiden (Khan & Dickerman.2006).

Women with Factor V Leiden disorder have an increased risk of miscarriage and stillbirth, some research suggests that it may also increase the risk of other complications during pregnancy, these complications may include pregnancy-induced high blood pressure (Preeclampsia), slow fetal growth, and early separation of the placenta from the uterine wall (placental abruption) (Kupferminc et al .1999). However, it is important to note that most women with the factor V Leiden mutation have normal pregnancies (Kupferminc et al .1999). Even among Caucasian populations, the association between the FVL mutation and RPL is still controversial (Hashimoto et al .1999).

1.5 .4 Diagnosis of inherited thrombophilia

The initial test for resistance to APC was based on the activated partial thromboplastin time (APTT) a clotting test measures the time it takes to make a clot, most commonly the APTT is performed with and without the addition of exogenous APC and the two clotting times expressed as a ratio (Dahlbäck .1995). Since then coagulation-based assays for the detection of APC resistance have been described and are commercially available, but the APC resistance ratios from some of these assay systems, however, have demonstrated overlap between unaffected individuals and individuals heterozygous for factor V Leiden (Hillarp. 1996).

DNA based assay

Unlike coagulation-based assays for APC resistance, DNA-based assays are not affected by pregnancy, the therapeutic use of anticoagulants, the use of oral contraceptives, or the presence of inhibitors such as lupus anticoagulants (Charles. et al .1998).

Three basic methods of DNA based assay are used:

1- A PCR-based assay that detects the guanine-to-adenine substitution at nucleotide 1691 by taking advantage of the loss of an *Mnl*I restriction site in the PCR product if the mutation is present (*Mnl*I Restriction Fragment Length Polymorphism (RFLP) (Bertina et al. 1994)..Another approach described by the same group was use of allele-specific hybridization (Bertina et al. 1994)

2- The use of primers that introduce a new restriction site if the mutation is present (Beauchamp et al. 1994).

3- The use of allele-specific primers for PCR amplification (Kirschbaum et al .1995 , Bellissimo et al. 1996).

1.5.5 Treatment of Thrombophilia (Factor V Leiden):

Treatment of FVL depends on a variety of conditions; such as whether it is caused by inherited or acquired clotting abnormalities. If you have only the abnormal factor V gene you do not need treatment unless your blood starts to clot. If you have or have had a blood clot and have the abnormal factor V gene, you will be put on anticoagulant therapy for a period of time ranging from 3 months to life. The length of time you are on anticoagulants depends on several issues such as; how many blood clots you have had, how serious your clot was and how many additional risk factors you may have. Protective anticoagulation therapy may be needed in times where your risk for developing a clot is greater such as pregnancy, surgery, or long plane/car rides.

Some women with thrombophilia might merit anticoagulant prophylaxis during their pregnancy and all or most that have a personal or family history of confirmed venous thromboembolism merit active thromboprohylaxis during the puerperium **(**Walker.2000).

Anticoagulant medications work by inhibiting or altering steps in the coagulation cascade. Medications such as warfarin (Coumadin, coumarins) are available in oral forms, whereas heparin and the low-molecular-weight heparins require either intravenous (IV) or subcutaneous (injections below the skin) routes for treatment.

Because coumarins cross the placenta, they have the potential to cause teratogenicity and fetal bleeding and should be avoided during pregnancy, although they can be used postpartum (Ginsberg et al .1998), Heparins do not cross the placenta and are safe for the fetus (Ginsberg et al .1989). Low molecular weight heparins have logistic advantages over unfractionated heparin and appear to be associated with a lower incidence of heparin induced Thrombocytopenia and Osteoporosis **(**Walker **.**2000). In a review of studies in which low molecular weight heparin was used in pregnancy, Sanson et al concluded that they are a safe alternative to unfractionated heparin as an anticoagulant during pregnancy (Sanson et al .1999).

Low-Molecular-Weight Heparins: are available as subcutaneous (below the skin) injections and have greater activity against factor Xa than thrombin , they have fewer bleeding complications that reflect their better bioavailability, longer half life, dose-independent clearance and decreased interference with platelets (Williams Obstetrics

2005).Low-Molecular-Weight Heparins used in serial venograms , were more effective than the unfractionated form in reducing thrombus size without increasing mortality or major bleeding complications (Williams Obstetrics 2005).

In the study by Gris et al, the live birth rate was 23 out of 80 women (29 %) in the aspirin group versus 69 out of 80 (86 %) in the enoxaparin group in patients with known thrombophilic predisposition (Gris et al .2004).

In another cohort study , patients with hereditary thrombophilia and RPL, 26 of the 37 pregnancies (70 %) in heparin-treated patients resulted in live births, compared with 21 of 48 (44 %) in untreated patients (OR 3.03; 95 %-CI: 1.12–8.36).The beneficial effect was seen mainly in women with no previous live births (OR 9.75; 95 %-CI: 1.59–52.48) (Carp et al .2003).

It seems obvious that heparin is the therapy of choice for prevention of recurrent miscarriage in patients with thrombophilia (Steinburg et al. 2009).

Heparin Antidote: For patients that have bleeding problems with heparin or require rapid neutralization of the heparin anticoagulation effect, Protamine sulfate is used. This is generally used only for severe bleeding **(**Walker**.** 2000).

Screening for factor V Leiden

Screening for inherited or acquired Thrombophilias is best limited to women with a personal or family history of venous thrombosis, early onset or recurrent pre-eclampsia, recurrent fetal growth restriction, unexplained fetal loss or stillbirth, and placental abruption (Bonnar et al.1999, and Lockwood.1999).

1.6 Literature Review

Since the turn of the last century, there has been extensive research focusing on both the genetic and acquired causes of thrombophilia, with particular focus on clotting events in the venous circulation. While there is evidence for adverse outcomes of pregnancy associated with thrombophilia, some studies ends with conflicting conclusions about the role of Factor V Leiden and Recurrent Pregnancy Loss.

A familial component of venous thrombosis was first recognized in the 1960s when reduced levels of AT were shown to be associated with recurrent thrombosis in a family (Egeberg . 1965) .The next step in finding other causes of inherited thrombophilia came later with the discovery of protein C deficiency (Griffin et al.1981)

Further confirmation of the multiple genetic factors for increased thrombotic risk came with the description of activated protein C-Resistance (APC-R) in 1993, and dramatically changed the diagnosis and management of venous thrombotic events. Dahlbäck et al described a large family from southern Sweden who demonstrated thrombosis in males and females throughout several generations and showed an autosomal dominant pattern of inheritance (Dahlbäck et al. 1993). The authors concluded that there was abnormality in the protein C/protein S regulatory system.

Subsequently, this abnormality was identified as a single amino acid substitution in one of the substrate proteins (Factor V) for activated protein C (APC) . This mutation was later characterized by Bertina and colleagues at the University of Leiden (Bertina et al. 1994). Subsequently as many as 15 % of the population in southern Sweden were identified as carriers of the factor V Leiden (FVL) gene (Dahlbäck et al. 1993).

Prevalence of Factor V Leiden in different populations

The prevalence of heterozygosity for the factor V Leiden mutation in Europeans, Israeli, Arabs, Canadian and Indian populations, ranges from 1 to 8.5 percent with most European studies reporting overall rates between 5 and 8 percent (Khan and Dickerman .2006).The prevalence is highest in Greece, Sweden, and Lebanon where it reaches about 15 percent in

some areas (Finan et al .2002). On the other hand, the mutation is apparently not present in African Blacks, Chinese, or Japanese populations (Ridker et al. 1998).

The Factor V Leiden Mutation and Recurrent Pregnancy Loss:

Several studies on the relationship between Factor V Leiden and recurrent abortion and other pregnancy complications were reported. Ridker published a study of 113 consecutive white American women with histories of 3 or more unexplained spontaneous abortions (Ridker et al. 1998). Sixteen of 437 (3.7%) women in the control group carried the factor V Leiden mutation, and 9 of 113 subjects (8%) tested positive for the mutation. These authors concluded that the factor V Leiden mutation may play a role in some cases of unexplained" recurrent pregnancy loss.

In another study by Souza et al examined 56 consecutive Brazilian women with 3 or more fetal losses, forty-six patients were "primary aborting,", ten patients were "secondary aborting,". Souza et al 1999.The factor V Leiden mutation was found in 4 of 56 patients (7.1%) and in 6 of 384 controls (1.6%). Again, an association between recurrent pregnancy loss and the factor V Leiden mutation was noted (Souza et al .1999).

Another investigation among Israeli women with unexplained recurrent abortions was done by Brenner et al.(1999)to study the role of three thrombophilic mutation including factor V Leiden, a case control study was held, 32% of cases had the mutation compared to 7% of controls, and they concluded that there was an association between factor V Leiden mutation and recurrent pregnancy loss (Brenner et al. 1999).

A case -control study done by Meinardi et al(1999), evaluated fetal wastage in 228 women with the factor V Leiden mutation and in 121 non affected controls, approximately 32% of carriers and 22% of non carriers experienced fetal loss, carriers had a 29.4% rate of miscarriage, and non carriers had a 17.4% rate, stillbirth rates were similar for both groups, (5.7% and 5.0% respectively) (Meinardi et al .1999). Carriers had a 10.1% risk for recurrence, and non carriers had a 4.1% risk. Homozygous patients had a greater risk for loss than heterozygous subjects did (Meinardi et al. 1999).
The association between factor V Leiden mutation and recurrent abortion was also studied among the Swedish women by Wramsby et al (2000) who examined 84 Swedish women

with at least 3consecutive, unexplained miscarriages (Wramsby et al .2000). Thirty-two women were secondary aborting, and 52 women were primary aborting. Thirteen of the 84 patients and 2 of 69 controls tested positive for the factor V Leiden mutation. Twelve (27.8%) of the primary-aborting women had the mutation, versus only 1 of the secondary-aborting women .Approximately 15% of the women with 3 or more miscarriages had the factor V Leiden mutation (Wramsby et al .2000).

A high prevalence of the factor V Leiden mutation was found among Lebanese women with recurrent idiopathic abortions mutation in a case control study conducted by Finan and coworkers, Factor V Leiden mutation was found among 41% of cases and among 16% of the control group. About 15.6% of the carrier cases were homozygous for the mutation and 84.4% were heterozygous, while all the carriers among the control group were heterozygous (Finan et al .2002).

Similar results were reported by Mahjoub et al who conducted a control case study among the Tunisian women to determine the prevalence of factor V Leiden mutation among women with 3 or more consecutive early, late and early and late recurrent abortions, these authors found that the prevalence was 27% of the Leiden mutation in the case group compared to 11.5% in the control group (Mahjoub et al .2005). Further more a study held by Martinelli et al. in Italy found that both factor V Leiden and the 20210G-A prothrombin mutation were associated with an approximate tripling of the risk of late fetal loss, approximately 10% of persons carrying the factor V Leiden mutation experience clinically significant thrombosis in their lifetime (Martinelli et al. 2000).

Moreover a case control study was carried to find the relation between factor V Leiden and venous thromboembolism by Gerhardt et al, in this study the authors examined 119 women with a history of venous thromboembolism during pregnancy and the puerperium and 233 age-matched normal women (Gerhardt et al. 2000). Among the women with a history of venous thromboembolism, a prevalence of factor V Leiden was 43.7%, as compared with 7.7% among the normal women (relative risk of venous thromboembolism, was 9.3) (Gerhardt et al .2000).

Other studies were conducted to investigate whether Factor V Leiden is more related with the first trimester or the second trimester abortion, Rai et al published a study that

associated APC resistance with second-trimester miscarriage, one hundred twenty English women with histories of recurrent abortions and no histories of thromboembolism were screened for APC resistance (Rai et al .1996). Seventy women had experienced only first trimester losses, and 50 women had experienced both first and second-trimester losses. The prevalence of APC resistance was higher in women with second-trimester miscarriages (10/50 - 20%) than women with first-trimester losses (4/70 -5.7%). The authors concluded that APC resistance may be responsible for second-trimester pregnancy loss (Rai et al .1996).

Similar results were found in another study conducted by Younis et al among Israeli women and showed that 6 % of the women with the recurrent first trimester abortion have the mutation compared with 22 % of women who had abortions in the second trimester. (Younis et al .2000). On the contrary Balasch et al did not find such association in a case control study included Spanish women with recurrent first trimester abortion and women with uncomplicated pregnancies, where the mutation was found in only one case and one control (Balasch et al. 1997).

Many other studies proved that obstetrical complications such as severe Preeclampsia, abruptio placentae, fetal growth retardation, and stillbirth are associated with intervillous or spiral-artery thrombosis and inadequate placental perfusion .In these studies was found a good relation between Factor V Leiden and these obstetrical complications such as the one held by Kupferminc et al in which they investigated 110 women who had 1 of these obstetrical complications and 110 women who had one or more normal pregnancies to determine the frequency of thrombophilic mutations, the R506Q mutation of factor V was detected in 22 of the women with obstetrical complications and in 7 of the women with normal pregnancies (Kupferminc et al. 1999).

Based on these findings the idea of general screening pregnant women for thrombophilia arose, however one study held by Greer stated that there is no evidence to support general screening of pregnant women for Thrombophilia (Greer .2000). Although, all pregnant women with a personal or family history of venous thromboembolism should be screened, screening should be extended to women with history of second-trimester pregnancy loss, severe or recurrent Preeclampsia, or intrauterine growth restriction, though the screening policy in a given country should be based on that country's own population data.

However, other studies suggested that because of the high prevalence of this mutation in certain populations positive effects associated with factor V Leiden have been postulated, possibly through human reproduction (Pauer et al. 2003; Hundsdoerfer et al., 2003; Morrison et al., 2002; Rai et al., 2001). Women who carry the factor V Leiden mutation lose less blood in menstruation; have higher hemoglobin levels, and possibly a lower incidence of life threatening post-partum hemorrhage which could be an evolutionary advantage (Lindqvist et al. 2001).

Surprisingly one study showed higher than expected prevalence of factor V Leiden mutation carriers was found in healthy pregnant women (9.2%) compared to general population figures (3%) (De Groot et al.1999+ Rosendaal et al.1995).A similar finding was reported in a recurrent miscarriage study where the prevalence of factor V Leiden was higher in women without a history of recurrent miscarriages (14 %) compared to those with recurrent miscarriages (1.7 %); (Dilley et al, 2002).

In an abstract reported by Roque et al, those investigators tested 377 patients with histories of recurrent first-trimester pregnancy loss for antiphospholipid antibodies, factor V Leiden mutation, prothrombin G20210A mutation, hyperhomocysteinemia, protein C deficiency, protein S deficiency, and antithrombin. These authors found no association between recurrent fetal wastage and thrombophilia (Roque et al. 2004) .Similar results were found by Dizon-Townson group in which they studied 40 couples with 3 or more idiopathic pregnancy losses ,the results indicated that none of the 40 women with idiopathic recurrent miscarriage tested positive for the factor V Leiden mutation (Dizon-Townson et al .1997**)**.

Furthermore a study held by Kutteh et al, those investigators evaluated 50 white women living in Tennessee who had had 3 or more pregnancy losses. One of the patients and 2 of the controls were heterozygous for the factor V Leiden mutation, the authors concluded that factor V Leiden mutation is not found at an increased frequency in women with recurrent early pregnancy losses (Kutteh et al .1999) .

In the Japanese population, a study was held by Hashimoto et al in which they studied 52 healthy Japanese women with histories of 3 or more consecutive unexplained first trimester miscarriages and 41 of their male partners. The results found none of the 52 women or the

41 partners carried the mutation. The control group had similar results. These authors concluded that factor V Leiden mutation is not associated with recurrent pregnancy loss in the Japanese population (Hashimoto et al .1999).

In a recent study done by Altintas and coworkers in south east Turkey, the prevalence of FV-Leiden mutation was 7.9% (9/114) in patient group, compared with 7% (13/185) in control group (p=0.780), one hundred and two patients were primary and 12 were secondary aborters, all FV-Leiden positive cases were primary aborters (8.8% ; 9/102, p=0.584).Authors reported that these results suggest that mutations have no role in etiology of first-trimester recurrent abortions (Altintas et al .2007)

Hypothesis

Although the Palestinian population is characterized by high fertility rate, it suffers from high rate of fetal loss due to many reasons. The high rate of fetal loss makes it necessary to investigate the different causes; so as to offer the best solutions and to overcome this problem . Eventually this will help to provide protective treatment and better observation of women at risk of this medical problem.

Objective

The main objective of this study was to determine the correlation between factor V Leiden mutation and Recurrent Abortion among Palestinian Pregnant Women In the west bank.

Chapter Two

Methodology

2.1 Data Collection

A retrospective case-control study was conducted among women with recurrent abortion (recurrent fetal losses in the three trimesters), especially designed questionnaire was developed to collect comprehensive clinical and personal information from all participating subjects.

Direct interview was used to collect data from cases and control groups. The questionnaire has been adopted from Shelbayeh work from An-Najah National University (Shelbayeh.2007).The questionnaire contained required information from all participants including: date of birth, age at marriage, result of the first pregnancy, gravidity, number of pregnancy losses, types of loss, medical history of the participants, use of drugs, if they have any pregnancy problems such as stillbirth or intra uterine fetal death (IUFD), intra uterine growth retardation (IUGR), babies born with low weight and whether they have a family history (including mother or sister) who suffered from recurrent abortion or other pregnancy complications (questionnaire form is shown later in the appendices)

2.1.1 Exclusion criteria:

- Women who are known to have chronic hypertension or diabetes .

- Women with anatomical abnormalities of the uterus.

- Women who are with known causes for recurrent miscarriages

2.1.2 Inclusion criteria:

Pregnancy losses were classified into three categories:

1-First trimester abortion (1st trimester) : pregnancy loss within the first 12 gestational weeks.
2- Second trimester abortion(2^{nd} trimester): pregnancy loss between the 12th and the 22^{nd} gestational week.

3-Third trimester pregnancy loss (IUFD): Fetal loss after the 22nd gestational week and is considered as Intrauterine Fetal Death (IUFD).

Women who had three or more of pregnancy losses (abortion) of unknown causes with normal blood pressure and no other health complication were included according to the following criteria :

1- Three or more first trimester abortions.

2- Two or more second trimester abortions

3- One or more third trimester pregnancy loss (IUFD).

The control group includes women who had two or more successful pregnancies with no health problems

2.1.3 Research Place:

The research was carried out in the Medical Research Centre at Al-Quds University, Abu Dies.

2.1.4 Ethical consideration:

The study was conducted in accordance with the Helsinki declaration, and approved by the Research and Ethical Committee at Alquds University.

2.2 Sample processing: Blood sample collection

Five ml venous blood specimens were collected from each participant into EDTA tubes from three centers :
- AL Hiba Center /Ramallah.
- Holy Family Hospital / Beithlehem
- Palestinian Medical Relief society Clinic / Jericho.

All samples were transported on ice to the Medical Research Centre, Al-Quds University ,for processing.

2.2.1 Genomic DNA Purification:

Genomic DNA was extracted using the Epicenter DNA purification Kit (the Master Pure TM Genomic DNA Purification Kit) according to the protocol described by the manufacturer ; as follows :

1- Five ml of blood were drawn into an EDTA tube.

2- Tubes were centrifuged at 1,000 x g for 15 minutes.

3- 300 ul of the Buffy coat were transferred to a 1.5 ml microcentrifuge tubes (eppendorf tubes), followed by vortexing.

4- 1.2 ml Lysis buffer 1(10mMTris-HCl,400 mM NACL and 2mM NA_2EDTA,pH 8.2) were added , tubes were inverted 3 times to mix , the bottom of the tubes were flicked to suspend any remaining material.

5- Tubes were incubated at room temperature for 5 minutes, and inverted 3 times to mix and flicked at the bottom then incubated for an additional 5 minutes , at the end of the second incubation ,tubes were inverted 3 times to mix and then were flicked at the bottom

6- Tubes were centrifuged for 25 seconds at 10,000 x g .

7- Most of the supernatant were removed, leaving approximately 25 ul of liquid; tubes were mixed to suspend the pellet (containing white blood cells).

8- 600ul of Lysis buffer 2 (10%SDS, protease K solution [1mg protease K in 1 % SDS and mM Na_2EDTA] were added, then the cells were pippetted up and down 5-7 times, 250 ul of the Precipitation Solution (6M NaCl) were added. to the mixture, the tubes were mixed vigorously by vortexing for > 30 seconds, followed by centrifugation for 10 minutes at 10,000 x g.

9- The supernatants were decanted into a clean microfuge tube, 700 ul of ice-cold isoprpanol were added. The tubes were inverted several times (30-40) times, stringy precipitates were seen.

10- Tubes were centrifuged at 4° C for 10 minutes at 10,000 x g..

11- The supernatants were carefully discarded without dislodging the pellets, 200 ul of Tris-EDTA (TE) Buffer (10mM Tris-HCL, 0.2 mM NA_2EDTA, pH7.5) were added.

12- The DNA in each tube was suspended by pipetting repeatedly followed by mixing for

13- seconds, or by incubation for overnight at room temperature.

14- Purified DNA was stored at -20° C until used.

2.2.2 Genomic DNA Qualification:

Genomic DNA was qualified by running on a 4-mm horizontal 1 % (w/v) agarose gel which was prepared in Tris-acetate-EDTA (TAE)buffer with the addition of 10 ul of 10mg/ml Ethedium Bromide . One ul of the extracted genomic DNA was mixed with 1.5 ul of 6x Loading buffer (and 9.5 ul)Tris-EDTA (TE) buffer (Promega).

A standard 100 bp ladder size marker (Invitrogen) was applied to the agarose gel as DNA size marker, each gel was allowed to run at constant voltage of 120 volts , visualized under UV light (Transilluminator UV Sigma 10B201413) and photographed using Fujifilm/gel documentation system / Ramco company-80-6247-61 / 007-11-0-70.

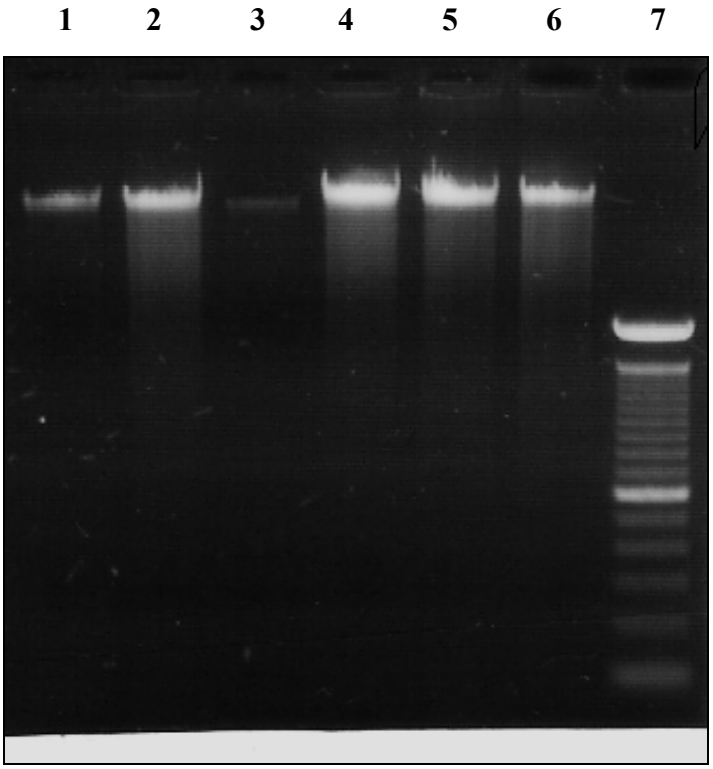


Figure 2.1 Agarose gel electrophoresis For Genomic DNA ;
Lanes 1-6 contain separate DNA samples (1 ul)
Lane 7 contains 100 Base Pair DNA as size marker ladder

2.3 Identification of Mutation

In order to identify the [R506Q] mutation in Factor V (factor V Leiden), the polymerase chain reaction (PCR) method was utilized with the amplification-refractory mutation system (ARMS).

Using patient genomic DNA as a template, a segment of exon 10 of factor V gene flanking nucleotide 1691 was amplified. PCR products were electrophoresed on 1.4 % Agarose gel and then visualized after ethedium bromide staining.

2.3.1 PCR & ARMS Test:

ARMS is based on the principle that the (Taq) polymerase enzyme lacks the 3'- 5' exonuclease activity and thus a mismatch between the 3' end of the PCR primer and the template will result in greatly reduced amplification efficiency. Hence, the presence of an amplified product indicates the presence of a particular allele and visa versa. ARMS test is an accurate, rapid and a simple method in which the amplification step and the diagnostic steps are combined (Bathelier et al .1998).

ARMS test is an amplification strategy based on the principle that the allele-specific oligonucleotide primers ASP1 and ASP2 are designed to be identical to the sequence of the two alleles over a region preceding the position of the variant nucleotide, up to and terminating in the variant nucleotide itself. ASP1 will bind perfectly to the complementary strand of allele 1 sequence permitting amplification with conserved primer. However, the 3'-terminal C of the ASP2 primer mismatches with the T of allele 1 sequence, making amplification impossible. Similarly ASP2 can bind perfectly to allele 2 and initiate amplification, unlike ASP1 . Figure 2.2 illustrates the ARMS test protocol.

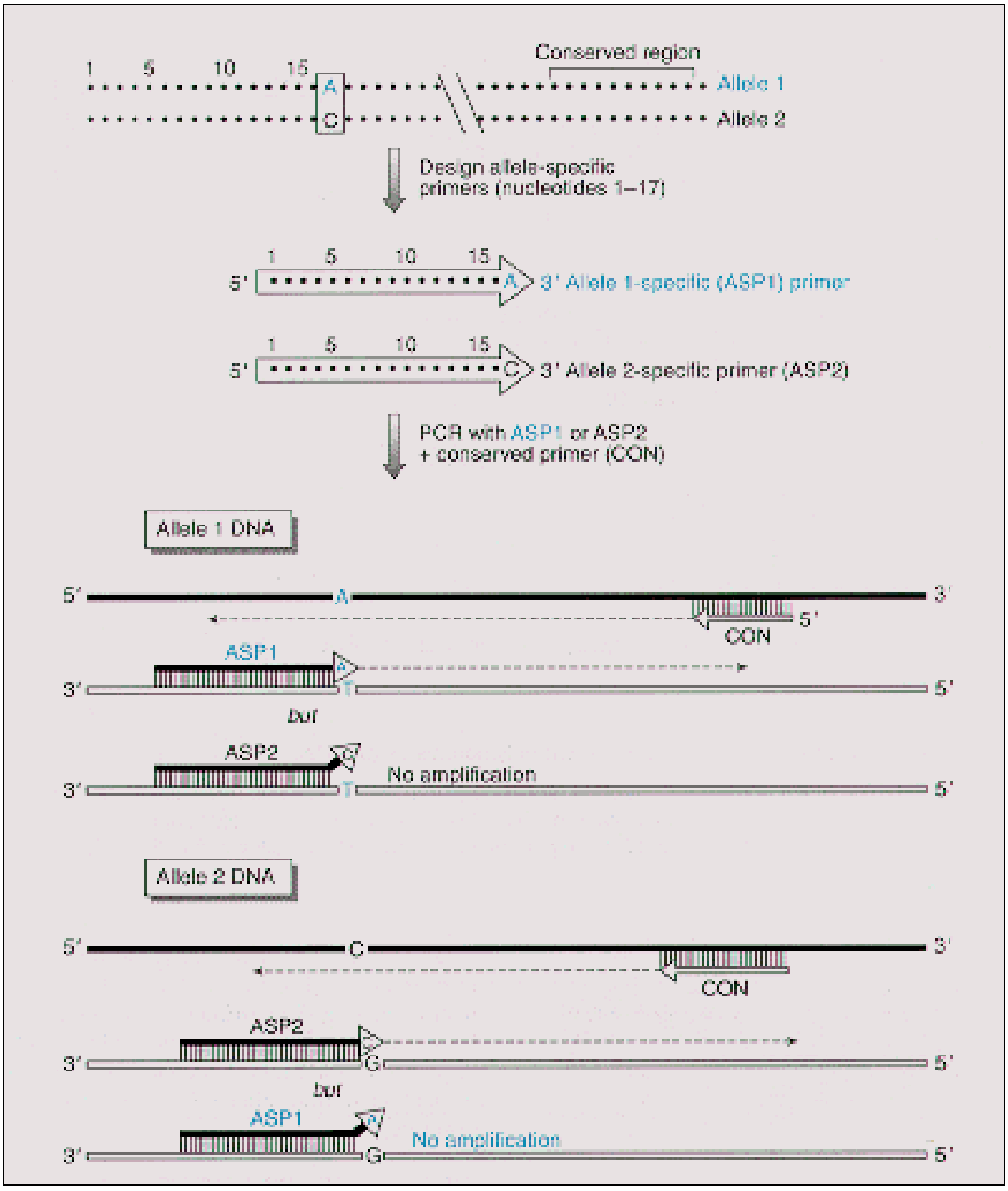


Figure 2.2 ARMS Test: Correct base-pairing at the 3' end of PCR primers is the basis of allele-specific PCR amplification .

2.3.2 Primer Sequence:

Genomic DNA was utilizes as the template for the PCR/ARMS amplification, one reaction with a common normal forward primer and a normal reverse primer (C & N) and the other with common normal forward primer and mutant reverse primers (C &M) as shown in table(2.1) .PCR primers were designed based on Exon 10 sequence of the human factor V gene. The mutant reverse primer had the 3' terminal nucleotide corresponding to the factor V mutation G→ A on the coding strand at position 1691, plus penultimate nucleotide mismatch (the mutant primer differs from the normal one at the last two 3' bases.) (Bathelier et al .1998) The primers sequences used for ARMS were as shown below in the table 2.1

Table (2.1) Primers Sequence

Primer type	Sequence
Common forward normal primer sequence (C) (nucleotide 120 – 140) of Exon 10	5'-ACATCTTAGAGTTTGATGA-3'
Normal reverse primer sequence (N) Correspond to the complementary strand of Exon 10 except the penultimate base at the 3' end was changed from T to G.	5'- GGACAAAATACCTGTATTCCGC-3'
Mutant reverse primer sequence (M)	**5'-**GGACAAAATACCTGTATTCCCT-3'

2.3.3 PCR mixture preparation:

PCR mixtures were prepared taking into account the number of the samples undergoing the reaction. Total volume of the mixture in each PCR tube was 25 ul.
The PCR mixtures were prepared according to the recipe listed below:

Table (2.2) PCR Mixture content

Reagents	Amount
10 X TakaRa buffer (final concentration)	2.5 ul (1X)
TakaRa d NTP mixture (2.5mM each)	2ul
Forward primer (0.15ug/ul) Invitrogen	1ul
Reverse primer (0 .15ug/ul) Invitrogen	1ul
Recombinant TakaRa Taq^{TM} DNA polymerase(5U/ul)	0.125 ul
DNA template(0.2ug/ul)	1ul
Steril Distilled water(autoclaved)	17.375
Total Volume	25ul

Two PCR reaction mixtures were prepared, one with Normal primer pair and the other with the mutant reverse primer. The PCR reactions were done in the Gene Amp® PCR thermocycler System 9700(AMIE.#80558120415).

Each PCR cycle consisted of 3 steps:

1- Denaturation step: in which the target DNA is incubated at high temperature ＞ 90° C to separate the two strands and make them accessible to hybridization by specific oligonucleotide primers.
2- Annealing step: in which the reaction mixture is cooled to a specific target temperature to allow the primers to anneal to their complementary sequences which depends on the primers melting temperature (TM) calculated to be 57° C .
3- Extension step: usually at 72 ° C (intermediate temperature) in which the primers are extended on the DNA template by the enzyme DNA polymerase.

A template, a 220 base pair segment of Exon 10 of the Factor V gene surrounding nucleotide 1691 was amplified using the following conditions:
Initial Denaturation (Hot start) at 95° C for 5 minutes.
Denaturation at 95° C for 30 seconds.
Annealing at 57° C for 30 seconds.
Extension at 72° C for 30 seconds.
A final extension step at 72° C for 5 minutes.
Number of cycles: 30 cycles.
The PCR products were analyzed by running on 1.4 % Agarose gel and results were recorded as Normal, Heterozygous or Mutant.

2.3.4 Preparation of PCR products for gel electrophoresis:

1.4 % (w/v) agarose gel was prepared in (TAE) buffer , 10 ul of the PCR reaction were mixed with 1.5-2 ul of 6x Loading buffer , the PCR products of each sample were applied to the gel in the order that the upper part of the gel contains the PCR product of normal PCR reaction while the lower part of the gel contains the PCR product of the mutant PCR reaction . One ul of DNA Ladder was applied to the agarose gel.

Each gel was allowed to run at 120 volts and later visualized under UV light (Transilluminator UV Sigma 10B201413) and photographed using Fujifilm/gel documentation system / Ramco company-80-6247-61 / 007-11-0-70.

All the results were read and recorded for each participant sample as following :

1- Participants with homozygous normal alleles yield one band in the normal PCR product reaction only (upper part)

2- Participants with heterozygous alleles yield two bands ; one in the normal PCR product reaction and the other in the mutant PCR product reaction.(upper & lower parts)

3- Participants with homozygous mutant alleles yield only one band in the mutant PCR product reaction .

2.4 Data Analysis

All the data including clinical informations about the participants and the genetic results of factor V gene were collected and analyzed using the SPSS version 12 (pss.Inc.2003) taking into consideration the followings:

Confidence interval of 95% was calculated in the study, P value of < 0.05 was considered for statically significance and the margin error of 5 % was accepted in the study.

This study provides a descriptive analysis of the main background and independent variables, across tabulation analysis to verify the level of statistical significance among different dependent and independent variables.

The following relationships represent the main areas of interest in the analysis and were considered in the results and discussion:

- Distribution of the participants according to the age and to the Body Mass Index (BMI).
- Number of pregnancies, deliveries and abortions among the participants.
- Distribution of different types of the first pregnancy outcomes.
- Distribution of different types of miscarriages among the case group, according to the type and the time of miscarriage.
- Distribution of factor V Leiden mutation among the participants.
- Distribution of factor V Leiden mutation among the different pregnancy outcomes in the case group in comparison to the control group.
- Prevalence of Thromboembolic events among relatives of participants.
- Prevalence of usage of oral contraceptive (OCPs) drugs among participants in the study.

Chapter Three

Results

General characteristics

Our case- control study included one hundred and four (104) participants; fifty (50) controls and fifty four (54) cases. The control group included women who had two or more successful pregnancies with no health problems of any sort. The case included women who had three or more pregnancy losses (abortion) of unknown causes, however women known to have chronic hypertension, anatomical abnormalities of the uterus, known causes for recurrent miscarriages and those who are known to have Leiden mutation, were excluded. The distribution of the participants as control and case groups is shown below in Figure 3.1

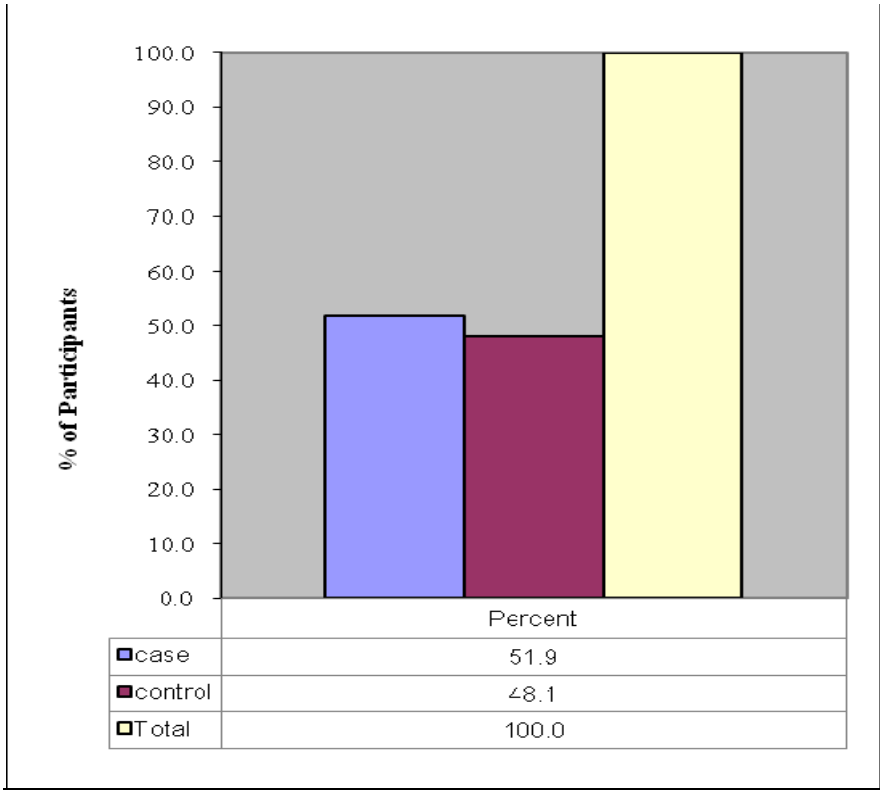


Figure 3.1: Percent distribution of Participants as Control and Case groups

3.2 Distribution of participants by age

The mean age of the case group was 30.7 years (range 19-53 years), while the mean age of the control group was 32 years (range 20-47 years) ,as summarized in table 3.2

Table 3.2 Distribution of participants by age

Group	Mean age	Range
Case	30.7	19-53
Control	32	20-47

3.3 Distribution of participants according to Body Mass Index (BMI):

Figure 3.3 demonstrates the distribution of participants according to BMI where 18< BMI <25 was considered normal, 25<BMI<30 was considered overweight and BMI >30 was considered obese.

In the control group, 42.7 % (21 woman) were normal and 57.3% (29 woman)were overweight or obese, while in the case group 34 % (18 woman) were normal and 66 % (35 woman) were overweight or obese .The data indicates that both groups show basically similar distribution.

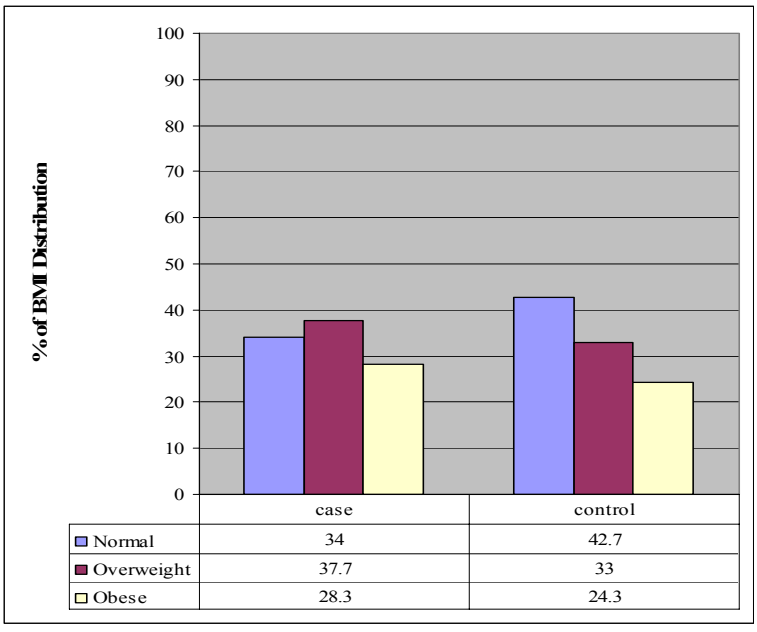


Figure 3.3: Percent distribution of participants according to BMI .

Obstetric History

3.4 Number of pregnancies, deliveries and abortions

Table 3.3 demonstrate that the total number of pregnancies among the case group was 419 pregnancies, with 285 abortions (68%) and 134 successful deliveries (32%), while the total number of pregnancies among the control group was 201 and all were successful. The overall percentage of abortion among all the participants was 46%.

Table 3.3 Number & Percentage of Pregnancies, Abortions and Deliveries among the participants

	Number & Percentage		
	Case	**Control**	**All**
Pregnancies	419	201	620
Abortions	285 (68%)	0 (0.0%)	285 (46%)
Deliveries	134 (32%)	201 (100%)	335 (54%)

These results are presented next in Figure 3.4

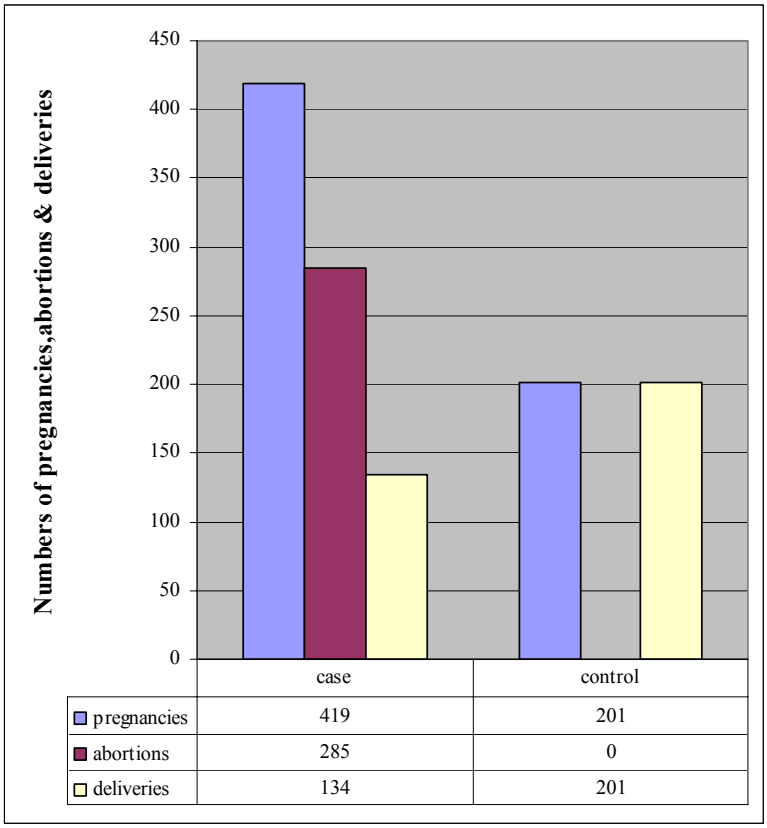


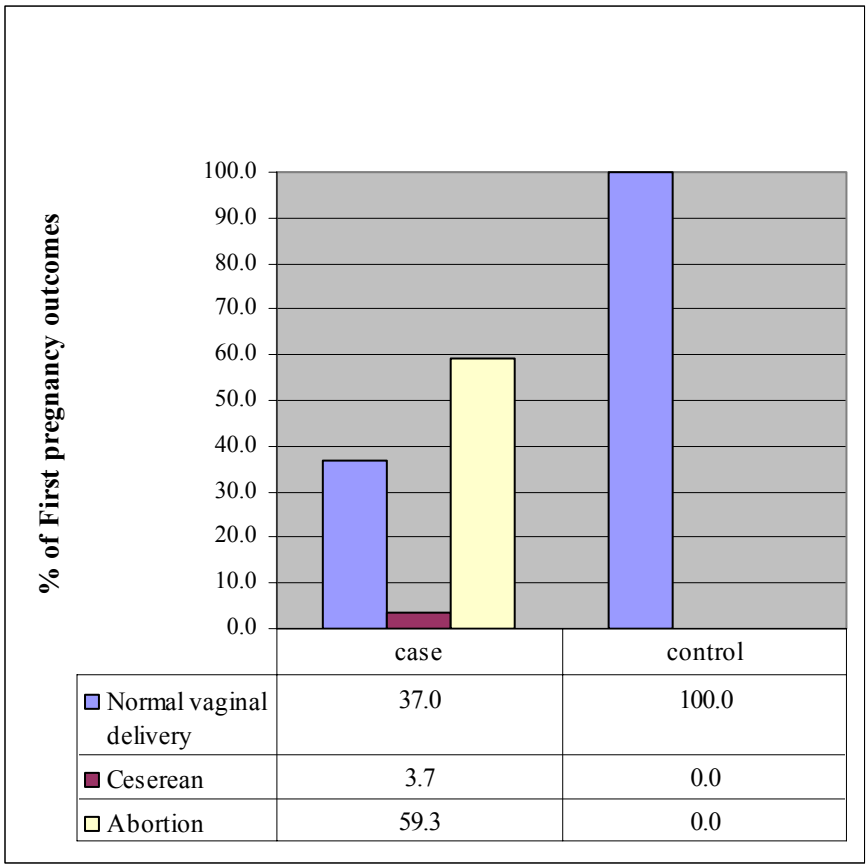
Figure 3.4: Number of Pregnancies, Abortions and Deliveries among all participants

3.5 First pregnancy outcome

Based on the result of the first pregnancy outcomes, this case study group was divided into two subgroups:

- **Primary abortion**: The result of the first pregnancy is abortion (no children before).
- **Secondary abortion**: Abortion occurs subsequent to having children; the result of the first pregnancy either normal or cesarean delivery.

Figure 3.5 demonstrates the distribution of different outcomes of the first pregnancy as follows: (37 %) 20 women in the case group and (100%) 50 women in the control group had normal delivery in the first pregnancy , while 3.7% (2 women's pregnancy) ended with cesarean section and 59.3% (32 women's pregnancy) ended with abortions, according to these results the percentage of the primary abortion was 59.3 % and that of the secondary abortion was 40.7 %.



	case	control
Normal vaginal delivery	37.0	100.0
Ceserean	3.7	0.0
Abortion	59.3	0.0

Figure 3.5: Percent distribution of adverse first pregnancy outcomes .

3.6 Distribution of different Types of Miscarriage between Cases

Four types of miscarriages accounted for all cases , these are illustrated graphically in Figure 3.6 as following:

- **Missed abortion**: The highest percentage 63.6% (164 abortion) in which the embryo or fetus died but not passed out of the uterus.
- **Bioclinical type (Bio):** 14.7 % (38 abortions) in which the pregnancy test give positive result while no fetus is growing.
- **A blighted ovum (Bligh)**: 20.5% (53 abortion), which happens when a fertilized egg attaches itself to the uterine wall, but the embryo does not develop.
- **Ectopic (Ecto):** 1.2% (3 abortion) in which the fertilized egg implants outside of the uterus**.** (these cases were excluded).

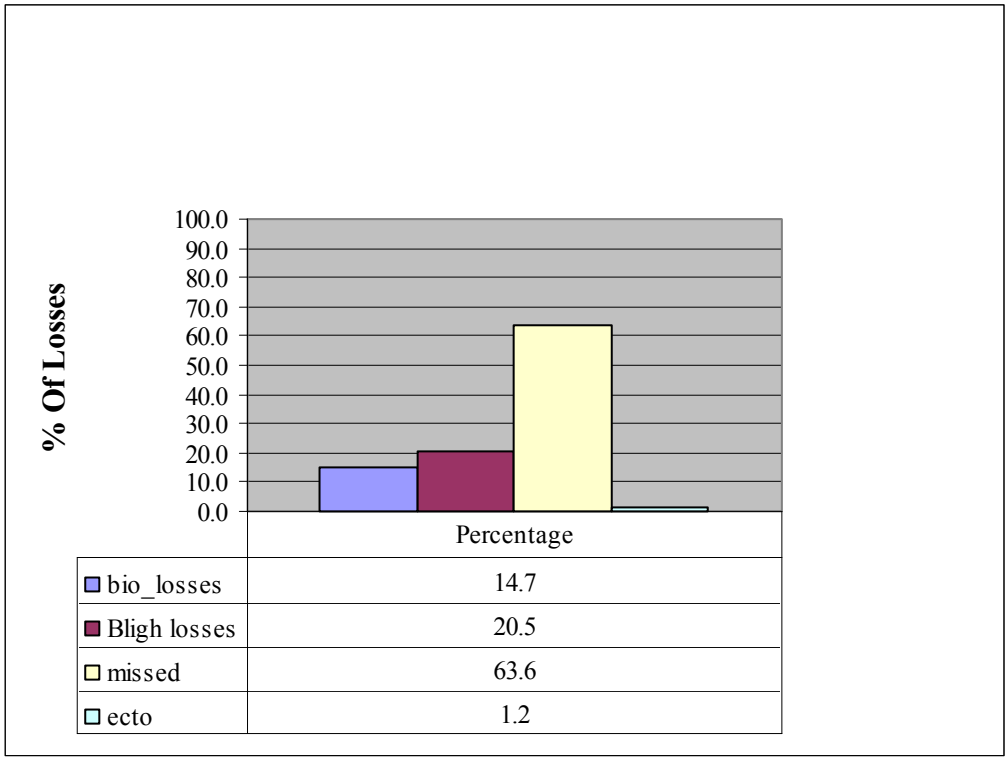


Figure 3.6 Percent Distribution of the different Types of Miscarriage among Cases.

3.7 Pregnancy Losses

Distribution of different pregnancy loss categories according to the number of aborters and the time of abortion was as shown in table 3.4 in which 53 women had suffered from 1st trimester abortion while only 1 woman had suffered from 2nd trimester abortion. Those women who had suffered from both types 1st trimester and 2nd trimester (12 woman) were categorized according to the number of abortions they had ; they were assorted as1st trimester whenever the number of abortions in the 1st trimester was higher than in the 2nd trimester abortion and visa versa .

Table 3.4 Distribution of pregnancy loss categories among the case group

Group	# of women	% of affected women
First trimester	53	98.15 %
Second trimester	1	1.85 %

3.8 Participants Factor V Genotype results

Participants factor V genotype was determined according to the gel electrophoresis for Leiden mutation PCR product analysis in which 220bp band appears in the gel (either in the upper or the lower or both) according to the factor V genotype as illustrated in figure3.8.1.

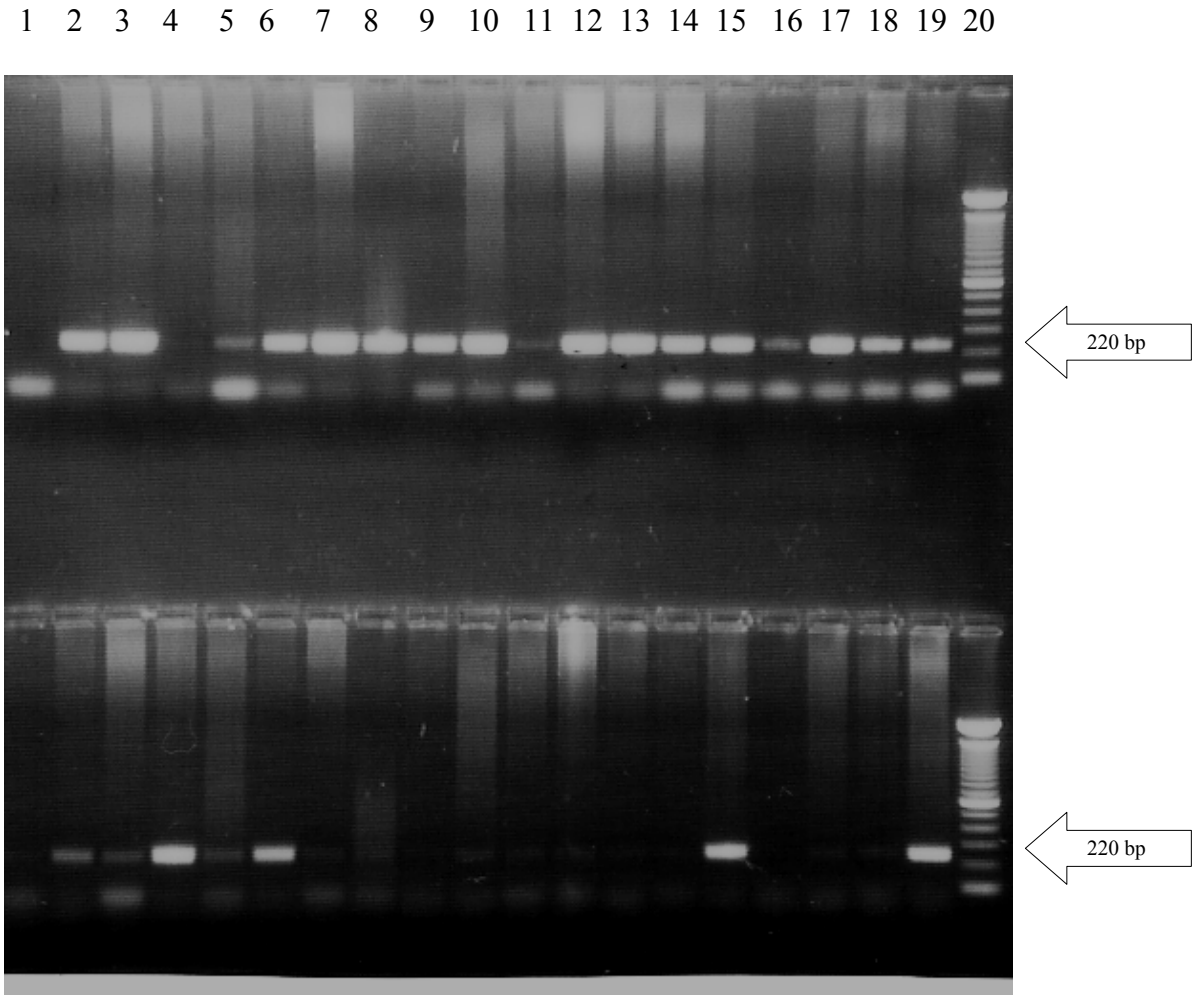


Figure 3.8 Gel electrophoresis for Leiden mutation PCR product analysis

PCR reaction products for 18 participants' samples:
The upper part includes PCR amplification products with normal primer while the lower part shows PCR reaction products with the mutant primer for the same samples.
Sample # 1: Control PCR reaction (omitting template DNA) to confirm the absence of contamination.
Samples # 7-14and 16 - 18 Amplified products are seen only with the Normal Primer and no amplification with the mutant primer indicating Homozygous Normal (both alleles are normal)
Samples # 2, 3, 5, 6, 15, 19; Amplification bands were detected both with the normal and mutant primers indicating heterozygous genotype (one normal and one mutant allele)
Sample # 4 : Amplification band is seen only with the mutant PCR primer indicating homozygous mutant genotype (both alleles are mutant).
Sample # 20: Size marker; DNA ladder (100 base pair ladder).

3.8.1 Distribution of Factor V mutation among participants :

According to the results of the gel electrophoresis of the PCR products distribution of Factor V genotype among participants was as follows:

The case group:

1- Normal alleles: 72.2 % (39 women).

2- Factor V Leiden mutation **:** 27.8 % (15 woman) ; distributed as 24.1% (13 woman) with heterozygous alleles and 3.7% (2 woman**)** with homozygous mutant alleles.

The control group:

1- Normal alleles: 78% (39 women).

2- Factor V Leiden mutation**:** 22 % (11 women) has heterozygous alleles & no homozygous alleles (the mutant gene) were found among the control group .

These results indicate the prevalence of factor V Leiden was not significantly higher among the case group than the control group (P value 0.324)

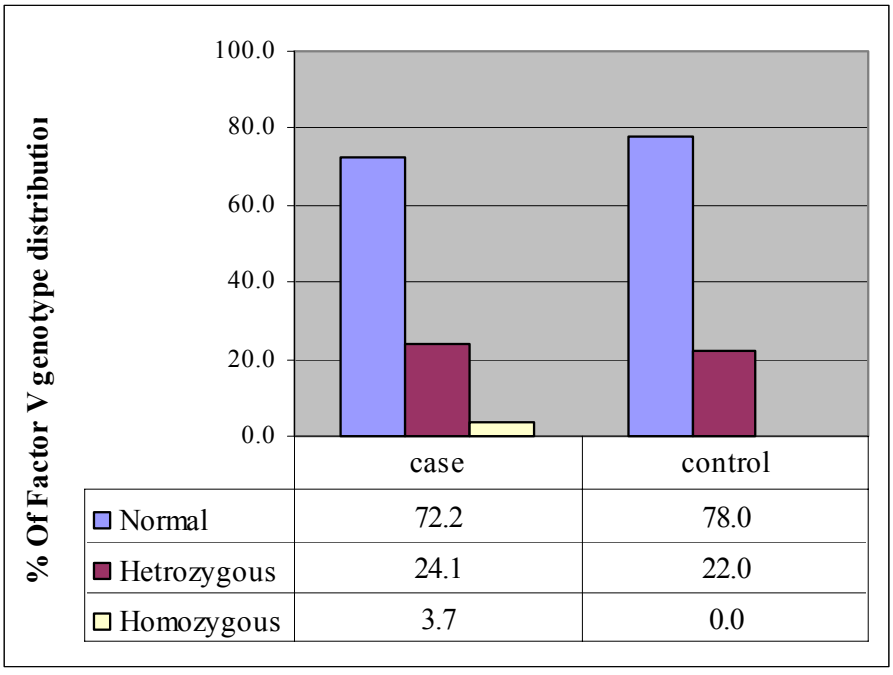


Figure 3.8 .1: Distribution of Factor V Leiden mutation among the participants

3.9 Distribution of factor V Leiden mutation among the different categories of abortion in comparison with control group.

First trimester aborters were more prevalent than second trimester aborters ,based on the genetic analysis presented in (Table 3.9) factor V Leiden mutation was more common among 1st trimester aborters than other categories. The distribution of Factor V Leiden mutation among the main aborters categories was as follows:

- First trimester abortion (28.3 % =24.5% heterozygous genotype and 3.7% homozygous mutant genotype).
- Second trimester abortion was = 100 % normal factor V genotype and 0.0 % factor v Leiden mutation genotype .

No mutation was detected among women with IUFD. Six women suffered from IUGR, but all were genotypically normal for Factor V Leiden mutation. Some women suffered from both types; First trimester and second trimester abortions (12 women), 33 % of them (4 woman) tested positive for Factor V Leiden mutation . (P value 0.015).

Table 3.9. Comparison of the distribution of Factor V Leiden mutation between the control group and the case group according to abortion trimester .

Group	# of women	% of affected women	% FVL mutation	p-value	Odd ratio
Control group	50	11	22 %		
1st trimester	53	15	28.3 %	0.306	1.4
2nd trimester	1	0	0 %	1.00	0

3.10 Prevalence of Thromboembolic events among relatives of participants

By comparing the information in the questionnaire filled by all participants about their relatives and their suffering from any kind of thromboembolic events, the results indicate that the percentage was significantly higher among the case group (18.5 %) in comparison to the control group (2 %); (P Value 0.006, Chi square value 7.490)

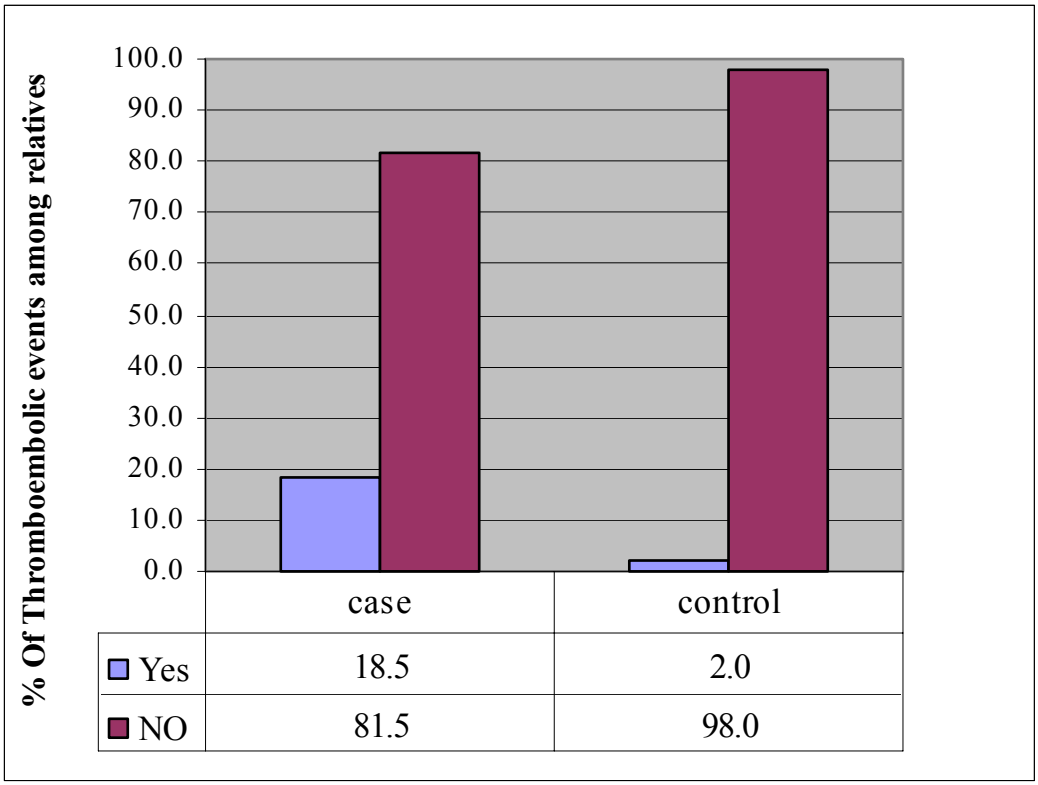


Figure 3.10 Prevalence of Thromboembolic events among relatives of all participants.

3.11 Prevalence of usage of oral contraceptive pills (OCPs) among participants in the study

The data indicates that the percentage of usage of the OCP drugs was slightly higher among cases (12.9%) than among the control group (8%) as shown in figure 3.11

These data indicates that the difference between the two groups is statistically insignificant (P value 0.416, chi square value 0.253)

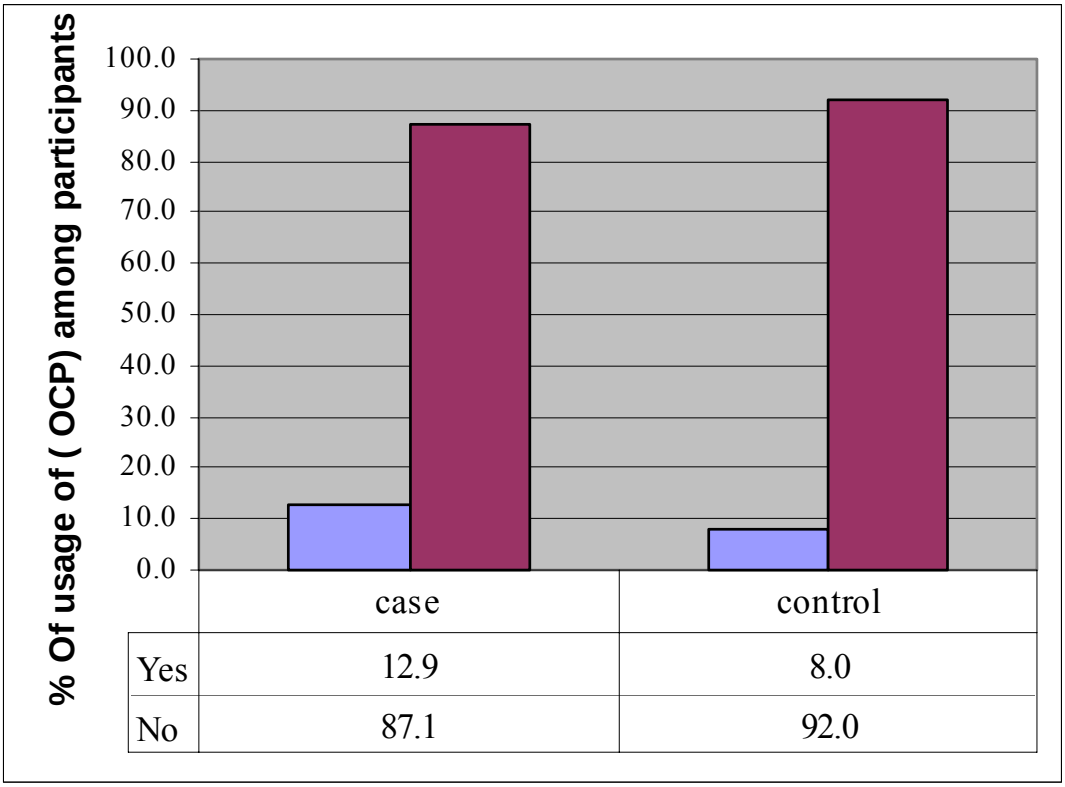


Figure 3.11 Prevalence of usage of Oral contraceptive pills (OCPs) drugs among all participants in the study.

Chapter Four
Conclusion and Recommendations

Giving a live birth is a very valuable event in the women's life; therefore, it is very important to avoid abortion which is considered a major problem in the world, the incidence of abortion is increasing in most developed and underdeveloped countries; occurring in about 20 % of all pregnancies and fewer than 50% of cases has definitive causes (Rees et al .1996). Thrombophilia may be a contributing factor in 40-60 % of unexplained multiple miscarriages (Brenner et al.1997). Thrombophilia, defined as the increased tendency of clot formation, is suggested to be one of the risk factors which contribute to the increasing incidence of poor pregnancy outcomes including recurrent abortion. (Cooper .1994)

Risk factors associated with thrombophilia are either acquired or inherited (Cooper .1994). Acquired factors include smoking, obesity, oral contraceptive pills, sedentary life, immobilization (especially in the postoperative period or after long bones fractures), pregnancy and other factors (Cooper . 1994). Inherited factors include deficiencies in protein S or protein C, mutations in some of the proteolytic cascade proteins, namely Factor II, Factor V and Methylene tetrahydrofolate reductase (Cooper .1994). Although a clear association has been established between fetal loss and certain thrombophilic states, such as antiphospholipid antibody syndromes, antithrombin deficiency, and combined defects, reports on the prevalence of inherited prothrombotic defects such as Factor V Leiden mutation and Methylene tetrahydrofolate Reductase C677T polymorphism in fetal loss are contradictory (Biswas et al .2008)

In the Palestinian community, the incidence of abortion is relatively high; reported to be 4-8% among women attending UNRWA antenatal care in the northern areas of the West Bank (Shelbayeh .2007), it is wroth to mention that this percentage is conservative; it differ from one area to another in Palestine (as shown earlier in my results). Therefore, exploring the relationship between Thrombophilias and poor pregnancy outcomes represents an important task in order to control fetal loss among pregnant Palestinian women.

The main aim of this study was to investigate the relationship between Factor V Leiden mutation and recurrent abortion among Palestinian women in order to minimize the chance of fetal loss through providing the appropriate management to those at high risk. In this study, one hundred and eighty (180) women from three medical centers in Ramallah, Beithlehem and Jericho were referred to us. From those only one hundred and four women were selected and seventy six women were excluded based on a specified inclusion criteria. The case group included women who had at least three times abortion with normal blood pressure and no other health complication, while the control group included women with good health and no pregnancy complications of any type and gave normal birth for at least two babies.

Since thrombophilia was suggested to be one of the risk factors that could increase the incidence of abortion, our work focused on investigating the contribution of factor V Leiden mutation as an inherited factor for thrombophilia on abortion .From the acquired factors our investigation focused on obesity and oral contraceptive pills. The data collected for these two factors indicates that both groups show basically similar distribution. Other factors, including smoking were not prevalent among participants in our study group.

Diagnosing thrombophilia was made using DNA-based assay (ARMS Test). This assay is more accurate than coagulation screening test because unlike coagulation screening test, DNA-based assays are not affected by pregnancy, therapeutic use of anticoagulants, use of oral contraceptives, or the presence of inhibitors such as lupus anticoagulants (Charles et al. 1998).Taking into consideration that Factor V Leiden is difficult to study because, although the mutation is fairly common in certain populations, it is rare in others, and more important, many affected patients do not have adverse results from the mutation (Wramsby et al. 2000).

The percentage of abortions among the case group was shown to be very high; reaching 68%, while all pregnancies in the control group ended successfully. The results of first pregnancy among the participants showed that all the pregnancies in the control group ended by normal vaginal deliveries, while in the case group 37% of first pregnancies ended with normal delivery, 3.7% of first pregnancies ended with cesarean section while 59.3% of first pregnancies ended with abortions. These results reflect the high incidence of abortion among the Palestinian population in comparison to the rate of abortion in

developed and underdeveloped countries totaling 20 %, keeping in mind that the 68% is among a group who are already suffering from recurrent pregnancy loss (RPL).

The prevalence of Factor V Leiden mutation was 27.8 % among the case group (24.1 % heterozygous and 3.7 % homozygous) and 22 % among the control group all are heterozygous for the mutant allele, this indicates that there is no significant difference in the total incidence of the Factor V Leiden mutation between the case and the control group (P value 0.324) except for the homozygous alleles in the case group.

BMI values showed basically similar distribution between the case and the control group, the usage of oral contraceptive pills (OCPs) among the case group (12.9%) was not significantly higher than that among the control group (8%) (P value 0.416). Meanwhile 14 % of the OCPs users among the case group were heterozygous while all the OCPs users among the control group were of normal genotype.

Intra uterine growth retardation (IUGR) was seen only in one woman of the control group and six women of the case group all with normal factor V genotype. Intra uterus fetal death (IUFD) was found in 6 women among the case group (11.1 %) with normal factor V genotype. No significance association between IUGR and IUFD and Factor V Leiden mutation is observed in this study, these results are in agreement with Lindoff et al (1997), Lindqvist et al (1998), and Kupferminc et al 1999, who concluded that although Factor V Leiden mutation and IUGR has been observed in association with APC resistance and Preeclampsia, IUGR prevalence did not correlate with APC resistance.

Comparing the distribution of the different categories of abortion, first trimester aborters was more prevalent than the second trimester aborters ; 53 women were first trimester aborters while only one woman was second trimester aborters ,also the prevalence of factor V Leiden genotype was more prevalent among the first trimester abortion (28.3 % =24.5% heterozygous genotype and 3.7% homozygous mutant genotype) than the second trimester (0.0 %) only one woman with normal genotype .Keeping in mind that several women have both types first and second trimester abortions. (12 woman 22%; 4 of them had Factor V Leiden Mutation 33%). On the contrary other studies, one done by Rai et al showed an association between APC resistance and second-trimester miscarriage (Rai et al .2000). Similarly ,Younis and coworkers showed that the prevalence of factor V Leiden mutation among cases with second trimester was higher than those with first trimester

abortion among Israeli women (Younis et al. 2000) .Balasch et al did not found such an association in a case control study among Spanish women with recurrent first trimester abortion (Balasch et l .1997).

Factor V Leiden mutation was more prevalent among secondary abortion (31.8% heterozygous) than primary abortion (25 % including 6.3% homozygous and 18.7% heterozygous). The main difference between the case and the control groups regarding Factor V Leiden mutation was the homozygous genotype of the mutation being only detected among the case group and absent in the control group. Moreover the different types of abortion were investigated in association with Factor V Leiden mutation. Most women in the case group suffered from more than one type of miscarriages. Some suffered from both first and second trimester abortions. Ectopic pregnancy (1.2%) was considered as abnormal pregnancy and was excluded from the study.

The missed type was the highest among first trimester abortion cases, which make sense keeping in mind that the first trimester period is very important in the embryo life. Definitely once pregnancy is established, survival of the fetus depends on placental development which is accomplished by the 17^{th} day after fertilization (Cleary – Goldman et al .2003). One study suggested that fetal carriers of the FVL mutation are at high risk during prenatal life (Dizon-Townson et al.1997). They explained that the fetomaternal circulatory system depends on both the mother and the fetus genotype, and thrombosis can occur in either maternal or fetal circulation. They found that the frequency of factor V Leiden mutation in miscarried fetuses is more than twice that in the general population (Dizon-Townson et al .1997). However, the main part of the placentary perfusion is encoded by both the maternal and the paternal genome (Toth et al .2008).

The fact that a fetus can inherit the mutation from either the maternal or the paternal gene pool, makes it obvious that the carrier status of the fetus, rather than that of the mother, may be the main consideration in fetal loss (especially with mothers of normal genotype) where it may cause thrombosis in the fetal circulation in the placenta (Cleary – Goldman et al. 2003).

A recent study conducted by Rashmi and colleagues on fetal gene defects and pregnancy failure in factor V Leiden mothers established a cause–effect relationship for the observed epidemiologic association between maternal FVL status and fetal loss and identified fetal

gene defects as risk modifiers of pregnancy failure in prothrombotic mothers (Rashmi et al.2007).These authors showed that pregnancy failure is mediated by Par 4-dependent activation of maternal platelets at the fetomaternal interface and likely involves a pathogenic pathway independent of occlusive thrombosis ,these results further demonstrate that the interaction between two given thrombosis risk factors produces markedly disparate consequences on disease manifestation (i.e., thrombosis or pregnancy loss), depending on the vascular bed in which this interaction occurs (Rashmi et al. 2007).

In a parallel study with similar investigations done in the northern areas of the West Bank , authors reported that the mutation was confirmed in 35 cases out of 137 (25.5%) and in 13 out of 155 controls (8.4%) (Shelbayeh . 2007). Contrary to our results they found a significant association between factor V Leiden and recurrent abortion. Factor V Leiden mutation was more prevalent in the second trimester than the first trimester. Table 4.1 below illustrates the main points of resemblance and differences between the two studies.

Table 4.1 Comparison between the study done in the northern areas and ours done in the central and southern areas of the West Bank

	Northern areas of West Bank	Central & southern areas of west Bank
Distribution of factor V Leiden	25.5%	27.8%
Heterozygous	Case: 23.3 Control :8.4%	Case: 24.1 % Control : 22%
Homozygous	Case:2.2 Control: 0 %	Case: 3.7% Control : 0%

The main resemblance between both studies was the prevalence of the heterozygous genotype in both the case group, while the homozygous genotype of the mutation was absent among the control group in both studies. In both studies Factor V Leiden was more prevalent among Secondary abortion than Primary abortion .It is worth to mention that among the group of women excluded from our study (76 women), 2 women were

homozygous and 13 were heterozygous. Therefore we suggest that it is important to follow up with these women in the future to see the status of their delivery trend.

The difference in the sample size due to differences in the inclusion criteria in both studies in the northern areas, e.g. pregnancy induced hypertension cases were included while in our case control study they were excluded. Still our sample size and negative results are comparable to those of the previous studies; the prevalence of *FVL* mutation in women with RSA and in a control group were 0/40 and 0/25 (Dizon-Townson et al.1997) and 10.7% (9/84) and 9.2% (8/87) (Pauer et al.1998) respectively.

A study done by Bettina and coworkers suggested that although recurrent miscarriage was not associated with paternal thrombophilia, abortions in the embryonic phase of fetal development were associated with a significantly higher incidence of maternal heterozygosity for FVL (Bettina Toth et al .2008).

Comparing our results with those of other studies done in other countries and populations, our results are in agreement with studies not supporting the association between Factor V Leiden and RPL such as those of Balasch et al among Spanish women in whom the mutation was found only in one case and one control (Balasch et al .1997). Another study done among a group of white women from Tennessee- America in which only one of the patients and 2 of the controls were heterozygous for the factor V Leiden mutation (Kutteh et al .1998). Further more Preston et al failed to establish a significant association between Factor V Leiden mutation and RPL among European women participating in the European Cohort on Thrombophilia (Cleary- Goldman et al .2003). Table 4.2 summarizes the results of these studies.

Table 4.2 Studies Not Supporting Association between Factor V Leiden Mutation and Recurrent Abortion

Author	Target Population	Prevalence Of Factor V Leiden Mutation		P-Value	Odds ratio 95%Confidence interval
		Cases (Frequency)	Controls (Frequency)		
Preston	European	26.9 (38/141)	23.5 (93/395)	0.04	0.9 (0.5-1.5)
Kutteh	American	2.0 (1/50)	4.0 (2/50)	0.49	(0.04-5.58)
Pauer	German	10.7 (9/84)	9.2 (8/87)	…(*)…	…(*)…
Dizon-Townson	American	0.0 (0/40)	0.0 (0/25)	…(*)…	…(*)…
Balasch	Spanish	1.8 (1/55)	2.0 (1/50)	…(*)…	(0.5-5.5)
Hashimoto	Japanese	0.0 (0/52)	0.0 (0/55)	...(*)…	…

(Cleary- Goldman et al 2003).(*)- The values were not calculated in the concerned references

Other studies reported a strong relationship between Factor V Leiden mutation and recurrent abortion; these studies are summarized in Table 4.3

Table 4.3 Studies supporting the association between the

Factor V Leiden and Recurrent Abortion

Author	Target Population	Prevalence Of Factor V Leiden Mutation		P-Value	Odds ratio 95%Confidence interval
		Cases (Frequency)	Controls (Frequency)		
Grandone	Southern Italian	16.3 (7/43)	4.2 (5/118) .	0.01	4.39 (1.3, 14.7)
Ridker	American	8.0 (9/113)	3.7 (16/437).	0.05	2.3 (1.0, 5.2)
Brenner	Israeli	32.0 (24/76)	10.0 (11/106)	<0.001	4.0 (1.8, 8.8)
Souza	Brazilian	7.1 (4/56)	1.6 (6/384)	…	4.9 (1.3, 17.8)
Meinardi	Scandinavian	31.6 (72/228)* 29.4 (67/228)†	22.3 (27/121)* 17.4 (21/121)†	0.06	2.12 (1.35, 3.33) 2.08 (1.33, 3.25)
Younis	Israeli	19.0 (15/78)	6.0 (8/139)	<0.05	3.17 (1.03, 9.8)
Wramsby	Swedish	15.5 (13/84)	2.9 (2/69).	0.0077 …	…(*)…
Foka	Greek	19.0 (15/80)	4.0 (4/100) .	0.003	5.5 (1.7, 17)
Reznikoff-Etievant	French	10.3 (27/260)	4.6 (11/240)	.018	2.4 (1, 5)
Cleary- Goldman et al 2003).(*)- The values were not calculated in the concerned references					

The controversial results in the previous studies may be due to the racial differences in the study populations. It is also possible that the frequency of the mutation may be concentrated in a population with potential thrombotic diseases, even though the basal prevalence in the general population is low. This Discrepancy can be explained based on the genetic background variation in different populations, many affected patients do not have adverse effect that result from Factor V Leiden mutation (Wramsby et al .2000).

Our investigations were performed only in female patients; we suggest that more investigations and studies should be done including couples (parental genes). More investigations and studies about the fetuses will give more insight on the recurrent abortion health problem, especially in deciding the right type of the anticoagulants that should be used in the treatment of the mother (whether it should cross the placenta or not and what are the possible effects in both cases?). For example aspirin may be advantageous, as it crosses the placenta and could perhaps protect against thrombosis on the fetal side of the maternal fetal circulatory system.

Although the Factor V Leiden mutation is one of many factors that may cause recurrent abortion, it is a good start to improve our knowledge about the array of factors suspected to play a significant role in recurrent abortion in order to participate in solving the recurrent abortion problem in our society.

In our case- control study no association between Factor V Leiden and recurrent pregnancy loss was shown, we think that other DNA assays should be done for those women suffering from recurrent abortion including genes encoding the natural anticoagulants like antithrombin, protein C, and protein S, which results in a loss of anticoagulant function. Definitely , further investigations concerning other genes related to recurrent pregnancy loss should be carried out similar to those done by Cyle S. Goodman et al in which they found that a panel of thrombogenic gene mutations including factor V G1691A, factor V H1299R (R2), factor II prothrombin G20210A, factor XIII V34L, β-fibrinogen -455G>A, PAI-1 4G/5G, HPA1 a/b(L33P), MTHFR C677T, and MTHFR A1298C to identify individuals at high risk for recurrent pregnancy loss (Cyle et al. 2006) . In addition to thrombin –receptor mutations and Vitamin K oxidation reduction complex (VKORC).

Recommendations

To the best of knowledge, this report is one of the first to describe the prevalence of the *FVL* mutation in women with recurrent pregnancy loss in the Palestinian population (south and center regions of the west bank). Data contribute to the clarification of the world wide distribution of the mutation in women with recurrent pregnancy loss (RPL). Since no association between Factor V Leiden and RPL was shown , we recommend the following:

1- More studies on other DNA assays should be done for those women suffering from recurrent abortion including the genes encoding the natural anticoagulants antithrombin, protein C, and protein S, which results in a loss of anticoagulant function. Only a large prospective study would enable a definite conclusion regarding this relation.

2- Increase public awareness about the importance of making the histopathology test for the abortuses in order to know the possible reasons for abortion, also the role of placental pathogenic mechanisms requires further evaluations.

In particular, one abortion is enough for each woman to start thinking of the causes and how to prevent it from happening again through medical consultation. any abortion should be reported in her file of Medical and Obstetrical history. Women of reproductive age should be given correct information concerning the risk of thromboembolic complications related to pregnancy, oral contraception and surgery, and obstetric complications related to thrombophilia.

References:

1. **Altintas, Abdullah; Pasa, Semir; Akdeniz, Nurten; Cil, Timucin; Yurt, Murat; Ayyildiz, Orhan; Batun, Sabri; Isi, Hilmi.(October 2007) "Factor V Leiden and G20210A prothrombin mutations in patients with recurrent pregnancy loss from the southeast of Turkey" .Annals of Hematology, Volume 86, Number 10, , pp. 727-731(5).**
2. **Arijit Biswas, Priya Choudhry, et al .(2008). "Recurrent Abortions in Asian Indians: No Role of Factor V Leiden Hong Kong/Cambridge Mutation and MTHFR Polymorphism". Clinical and Applied Thrombosis/Haemostasis, Vol. 14, No. 1, 102-104 .**
3. **Balasch J, Reverter JC, Fabregues F, et al. (1997)."First-trimester repeated abortion is not associated with activated protein C resistance". Human Reprod,12.1094-1097.**
4. **Bathelier C, Champion T, and Lucotte G.(1998)." ARMS test for diagnosis of factor V Leiden mutation and allele frequencies in France". Molecular and Cellular Probes ,12.121-123.**
5. **Bellissimo DB, Kirschbaum NE, Foster PA.(1996)" Improved method for factor V Leiden typing by PCR-SSP". Thromb Haemost.75:520.**
6. **Benirschke. (1998)."Remarkable Placenta" .Clin Anat. 11:194-205.**
7. **Bertina RM, Koeleman BP, Koster T, et al.(1994) ."Mutation in blood coagulation factor V associated with resistance to activated protein C". Nature. ,369:64-67.**
8. **Bettina Toth [1,*], Franziska Vocke [1,*], Nina Rogenhofer [1] , Klaus Friese [1] , Christian J. Thaler [1] , Peter Lohse [2].(2009) "Paternal Thrombophilic Gene Mutations Are Not Associated with Recurrent Miscarriage".**
9. **Bonnar J, Green R, Norris L.(1999)." Perinatal aspects of inherited thrombophilia". Semin Thromb Hemost ,25(5):481-5.**
10. **Brenner B, Mandel H, Lanir N, Younis J, Rothbart H, Ohel G, et al. (1997)."Activated protein C resistance can be associated with recurrent fetal loss". Br J Haematol , 97.551-4.**
11. **Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N.(1999). "Thrombophilic polymorphisms are common in women with fetal loss without apparent cause". Thromb Haemost. ,82:6-9.**

12. **Carp H, Dolitzky M, Inbal A. (2003)."Thromboprophylaxis improves the live birth rate in women with consecutive recurrent miscarriages and hereditary thrombophilia". J Thromb Haemost , 1. 433–8.**
13. **Chan Wp Chan WP, Lee CK, Kwong YL, Lam CK, Liang R.(1998 Feb 15)." A novel mutation of Arg306 of factor V gene in Hong Kong Chinese". Blood 91(4):1135-9.**
14. **Christopher et al .**(1998) ."**Circulating markers of oxidative stress are raised in normal pregnancy and pre-eclampsia" .**
15. **Clark P, Brennand J, Conkie JA, et al.(1998)." Activated protein C sensitivity, protein C, protein S and coagulation in normal pregnancy". Thromb Haemost ,79.1166 .**
16. **Cooper D.N.(1994)." The molecular genetics of familial venous thrombosis". Bailliere′s Clin Haematol.,7.637-674.**
17. **Cyle S. Goodman [1] , Carolyn B. Coulam [1,2] , Rajasingam S. Jeyendran [3] , Vida A. Acosta [3] , Roumen Roussev [1].(2006)." Which Thrombophilic Gene Mutations are Risk Factors for Recurrent Pregnancy Loss?". A merican Journal of reproductive immunology Volume 56 Issue 4. 230 – 236** .
18. **Dahlback B. (1995)."Resistance to activated protein C, the Args06 to Gln mutation in the factor V gene, and venous thrombosis". Thrombos Haemostas , 73: 739-742.**
19. **Dahlbäck B, Carlsson M, Svensson PJ. (1993)." Familial Thrombophilias due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C". Proc Natl Acad.Sci.USA, 90:1004-1008.**
20. **David Keeling .(N.D)" The Discovery of Resistance to Activated Protein C and Identification of the mechanism".**
21. **Donna Dizon-Townson, MD, Connie Miller, PhD, Baha Sibai, MD, et al.(2005).**"The **Relationship of the Factor V Leiden Mutation and Pregnancy Outcomes for Mother and Fetus ". Obstetrics & Gynecology ,106:517-524.**
22. **De Groot CJM, Bloemenkamp KWM, Duvekot EJ, Helmerhorst FM, Bertina RM, Van Der Meer F, De Ronde H, Oei SG, Kanhai HHH, Rosendaal FR.((1999)."Preeclampsia and genetic risk factors for thrombosis: a case control study". Am J Obstet Gynecol, 181:975-980.**
23. **Dilley A., Benito C., Hooper W.C., Austin H., Miller C., El-Jamil M., Cottrell S., Benson J., Evatt B.L.,Patterson-Bamett A., et al. (2002) "Mutations in the factor**

V, prothrombin and MTHFR genes are not risk factors for recurrent fetal loss". J. Mat. Fet. Neonat. Med., 11: 176-182

24. **Dizon-Townson DS, Meline L, Nelson LM, Varner M, Ward K. (1997)."Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction". Am J Obstet Gynecol. ,177:402-405.**

Dizon-Townson DS, Nelson LM, Jang H, Varner MW, Ward K.(1997)." The incidence of the factor V Leiden mutation in an obstetric population and its relationship to deep vein thrombosis". Am J Obstet Gynecol,176:883-886.

Drucilla Roberts,Robert S . Schwartz .(2002)."Clotting and Hemorrhage in the Placenta- A Delicate Balance".

27. **Egeberg O.(1965)." Inherited antithrombin deficiency causing thrombophilia ".Thromb Diath Haemorrh. ,13:516–530.**
28. **Esteban González Burchard, M.D., Elad Ziv, M.D., Natasha Coyle, Ph.D., et al .(2003) ." The Importance of Race and Ethnic Background in Biomedical Research and Clinical Practice".**
29. **FinanR, TamimH, AmeenG,etal. (2002)."Prevalence of FactorVG1691A(factorV-Leiden)and Prothrombin gene mutations in a recurrent miscarriage population". American Journal of Hematology , 71.300-305.**
30. **Foka ZJ, Lambropoulos AF, Saravelos H, et al.(2000)." Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. Hum Reprod , 15.458-462.**
31. **Gerhardt A, Scharf RE, Beckmann MW, et al. (2000)."Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium".**
32. **Gerry A.F. Nicolaes; Björn Dahlbäck.(2002)."Description of a Janus-Faced Protein ;Factor V and Thrombotic Disease".**
33. **Ginsberg JS, Hirsh J. (1998)."Use of Antithrombotic agents during pregnancy". Chest , 114.524S–30S.**
34. **Ginsberg JS, Kowalchuk G, Hirsh J, et al.(1989)." Heparin therapy during pregnancy: risks to the fetus and mother". Arch Intern Med ,149.2233.**
35. **Guasch, J. F.; Lensen, R. P. M.; Bertina, R. M. (1997)." Molecular characterization of a type I quantitative factor V deficiency in a thrombosis patient that is 'pseudo homozygous' for activated protein C resistance". Thromb. Haemost, 77.252-257.**

36. **Grandone E, Margaglione M, Colaizzo D, d'Addedda M, Cappucci G, Vecchione G. (1997)."Factor V Leiden is associated with repeated and recurrent unexplained fetal losses". Thromb Haemost,77.822-4.**
37. **Greengard JS, Eichinger S, Griffin JH, Bauer KA. (1994)."Variability of thrombosis among homozygous siblings with resistance to activated protein C due to an Arg-to-Gln mutation in the gene for factor V". N Engl J Med ,331.1559-1562**
38. **Greer IA. (2000)."The challenge of thrombophilia in maternal-fetal medicine". N Engl J Med ,342.424-5.**
39. **Griffin JH, Evatt B, Zimmerman TS, Kleis AJ, Wideman C.(1981)." Deficiency of protein C in congenital thrombotic disease". J Clin Invest , 68.1370-1373.**
40. **Gris JC, Mercier E, Quéré I, Lavigne-Lissalde G, Cochery-Nouvellon E, Hoffet M, Ripart-Neveu S, Tailland ML, Dauzat M, Marès P.(2004)."Low-molecular-weight heparin vs. low-dose aspirin in women with one fetal loss and a constitutional thrombophilic disorder". Blood , 103. 3695–9.**
41. **Hashimoto K.Shizusawa Y.Shimoya K.et al.(1999)."The factor V Leiden mutation in Japanese couples with recurrent spontaneous abortion". Human reproduction ,14.1872-1874.**
42. **Hillarp A, Zöller B, Dahlbäck B.(1996)." Activated protein C resistance as a basis for venous thrombosis". Am J Med , 101.534-540.**
43. **Hundsdoerfer P., Vetter B., Stover B., Bassir C., Scholz T., Grimmer I., Monch E., Ziemer S., Rossi R., Kulozik A.E.(2003)." Homozygous and double heterozygous Factor V Leiden and Factor II G20210A genotypes predispose infants to thromboembolism but are not associated with an increase of foetal loss. Thromb.Haemost,90(4):628-635**
44. **Isobel D Walker[1] .(2000)."Thrombophilia in pregnancy " Clin Pathol , 53:573-580.**
45. **Jane Cleary-Goldman, MD; Gary S. Nakhuda, MD; Ralf C. Zimmermann, MD; Mark V. Sauer, MD.(2003)." The Role of Factor V Leiden Mutation in Recurrent Pregnancy Loss".**
46. **Judith S. Greengard, Sabine Eichinger, John H. Griffin, and Kenneth A. Bauer .(1994)" Factor V Leiden and Thrombophilia". Engl J Med December 8, 331(23):1559-1562**
47. **Katherine A. Hajjar, M.D.(1994). Factor V Leiden - An Unselfish Gene?**

48. **Kirschbaum NE, Foster PA. (1995)."The polymerase chain reaction with sequence specific primers for the detection of the factor V mutation associated with activated protein C resistance". Thromb Haemost ,74:874-878.**
49. **Koster T, Rosendaal FR, de Ronde H, et al. (1993)."Venous thrombosis due to a poor anticoagulant response to activated protein C".Leiden thrombophilia study. Lancet ,342:1503–6.**
50. **Kupferminc MJ, Eldor A, Steinman N, et al. (1999)."Increased frequency of genetic thrombophilia in women with complications of pregnancy". N Engl J Med ,340:9-13.**
51. **Kutteh WH, Park VM, Deitcher SR.(1999)." Hypercoagulable state mutation analysis in white patients with early first-trimester recurrent pregnancy loss". Fertil Steril, 71:1048-1053.**
52. **Laurent O. Mosnier and John H. Griffin .(2006)." Protein C anticoagulant activity in relation to anti-inflammatory and anti-apoptotic activities".Frontiers in Bioscience September 1,11.2381-2399.**
53. **Lindoff C, Ingemarsson I, Martinsson G, Segelmark M, Thysell H, Astedt B.(1997)." Preeclampsia is associated with a reduced response to activated protein C". Am J Obstet Gynecol ,176.457-60.**
54. **Lindqvist PG, Svensson PJ, Dahlback B, Marsal K . (2001)."Factor V Q506 mutation (activated protein C resistance) associated with reduced intrapartum blood loss--a possible evolutionary selection mechanism". Thromb Haemost ,79:69-73**
55. **Lockwood CJ.(1999)." Heritable coagulopathies in pregnancy". Obstet Gynecol Surv ,54(12):754-65**
56. **Lutz Charles T. Lutz, Paul A. Foster et al .(1998)."Multicenter Evaluation of PCR Methods for the Detection of Factor V Leiden (R506Q) Genotypes". Clinical Chemistry,44:1356-1358.)**
57. **MahjoubT,Mtiraoui N ,Tamim H et al . (2005)."Adverse pregnancy outcomes and maternal factor V (Leiden) and Prothrombin G20210 genotype in women with a history of recurrent idiopathic miscarriages ."American Journal of Hematology ,80:12-19.**
58. **Martinelli I,EmanuelaT,CetinI,etal. (2000)."Mutations in coagulation factors in women with unexplained late fetal loss ".**

59. **März W, Nauck M, Wieland H. .(2000)."The molecular mechanisms of inherited thrombophilia".**
60. **Meinardi JR, Middeldorp S, de Kam PJ, et al. (1999)."Increased risk for fetal loss in carriers of the factor V Leiden mutation". Ann Intern Med,130:736-739.**
61. **Meinardi, J. R.; Pelsma, P. M.; Koning, H.; van der Meer, J.; Middeldorp, S.; Buller, H. R.; Hamulyak, K. (1997)." Molecular characterization of a type I quantitative factor V deficiency in a thrombosis patient that is 'pseudo homozygous' for activated protein C resistance". Thromb. Haemost, 77: 252-257.**
62. **Morrison E.R., Miedzybrodzka Z.H., Campbell D.M., Haites N.E., Wilson B.J., Watson M.S., Greaves M., Vickers M.A. (2002)."Prothrombotic genotypes are not associated with pre-eclampsia and gestational hypertension: results from a large population-based study and systematic review". Thromb. Haemost, 87: 779-785**
63. **Pauer H.U., Voigt-Tschirschwitz T., Hinney B., Burfeind P., Wolf C., Emons G., Neesen J.(2003)." Analyzes of three common thrombophilic gene mutations in German women with recurrent abortions". Acta Obst. Gynecol. Scand, 82: 942-947. 73.**
64. **Pauer HU, Neesen J, Hinney B.(1998)." Factor V Leiden and its relevance in patients with recurrent abortions". Am J Obstet Gynecol, 178:629.**
65. **Price DT, Ridker PM.**(1997)." **Factor V Leiden mutation and the risks for thromboembolic disease: a clinical perspective". Ann Intern Med, 127:895-903.**
66. **Pildner von Steinburg S, Schneider KTM .(2009)."Recurrent Spontaneous Abortions .An Update on Diagnosis and Management". J. Reproduktionsmed. Endokrinol , 6 (1), 11-16.**
67. **Rashmi Sood[1], Mark Zogg[1], Randal J. Westrick[2], Yi-he Guo[1], Edward J. Kerschen[1], Guillermina Girardi[3], Jane E. Salmon[3], Shaun R. Coughlin[4], and Hartmut Weiler[1].(2007)." Fetal gene defects precipitate platelet-mediated pregnancy failure in factor V Leiden mothers ". The Journal of Experimental Medicine, Vol. 204, No. 5, 1049-1056 .**
68. **Rai R, Regan L. (2000)."Thrombophilia and adverse pregnancy outcome". Semin Reprod Med. 18:369-377.**
69. **Rai, R., Regan, L., Hadley, E. et al. (1996)."Second-trimester pregnancy loss is associated with activated C resistance". Br. J Haematol, 92, 489–490.**

70. **Rai R., Shlebak A., Cohen H., Backos M., Holmes Z., Marriott K., Regan L.(2001)."Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage". Hum.Reprod, 16,961-965.**
71. **Rees DC.The population genetics of Factor V Leiden (Arg 506Glu)BR J Haematol.1996;95:579-586**
72. **Rees DC, Cox M and Clegg JB. (1995)."World distribution of factor V Leiden". Lancet , 346: 1 1 33-1 1 34.**
73. **Rosendaal Frits R. (2005)."Venous Thrombosis: The Role of Genes, Environment, and Behavior" .The American Society of Hematology .**
74. **Rosendaal FR, Koster T, Vandenbroucke JP and Reitsma PH. (1995)."High risk of thrombosis in patients homozygous for Factor V Leiden (Activated protein C resistance). Blood , 85: 1504-1508.**
75. **Ridker PM, Miletich JP, Buring JE, et al. Factor V Leiden mutation as a risk factor for recurrent pregnancy loss. Ann Intern Med. 1998;128:1000- 1003.**
76. **Ridker PM, Miletich JP, Hennekens CH, Buring JE.(1997)." Ethnic distribution of factor V Leiden in 4047 men and women: implications for venous thromboembolism screening". JAMA**
77. **Roqué H., Paidas M.J., Funai E.F., Kuczynski E., Lockwood C.J.(2004)." Maternal thrombophilias are not associated with early pregnancy loss". Thromb. Haemost,91(2):290-5 .**
78. **Salwa Khan and Joseph D Dickerman .(2006)." Hereditary thrombophilia".**
79. **Sanson B-J, Lensing AWA, Prins MH, et al. (1999)."Safety of low molecular weight heparin in pregnancy: a systematic review". Thromb Haemost ,81:668–72.**
80. **Shen L, Dahlback B.(1994)." Factor V and protein S as synergistic cofactors to activated protein C in degradation of factor VIIIa". J Biol Chem ,269:18735-18738.**
81. **Shelbayeh, K(2007):Relationship between Factor V Leiden and Poor Pregnancy Outcomes .An-Najah National University, Palestine (Unpublished Thesis)**
82. **Shen MC, Lin JS, Tsay W. (1997)."High prevalence of antithrombin III, protein C and protein S deficiency, but no factor V Leiden mutation in venous thrombophilic Chinese patients in Taiwan". Thromb Res ,87:377-**

83. **Souza SS, Ferriani RA, Pontes AG, Zago MA, Franco RF.(1999)." Factor V Leiden and factor II G20210A mutations in patients with recurrent abortion". Hum Reprod,14:2448-2450.**

84. **SPSS Inc.(2003): SPSS 12 for windows - Statistical package for the social sciences. Chicago, USA. (www.spss.com).**

85. **Tony L. Yang, Jisong Cui, Alnawaz Rehumtulla, Angela Yang, Micheline Moussalli, Randal J. Kaufman, and David Ginsburg,(1998)."The Structure and Function of Murine Factor V and Its Inactivation by Protein C". Blood, Vol. 91 No. 12 (June 15), pp. 4593-4599**

86. **Tracy PB, Eide LL, Bowie EJ. (1982)."Radioimmunoassay of factor V in human plasma and platelets". 1982; 60: 59–63.**

87. **Williamson, D,et al (2005):Williams Obstetrics ,volume II, chapter 47, Thromboembolic disorders - p. 1073.**

88. **Williamson D, Brown K, Luddington R, Baglin C, Baglin T.(1998)."Factor V Cambridge: a new mutation (Arg306 – Thr) associated with resistance to activated protein C". Blood, Feb 15 .91(4):1140-4.**

89. **Wilson DB, Salem HH, Mruk JS, Maruyama I, Majerus PW. (1984)."Biosynthesis of coagulation factor V by a human hepatocellular carcinoma cell line". J Clin Invest , 73: 654–658.**

90. **Wramsby ML, Sten-Linder M, Bremme K.(2000)." Primary habitual abortions are associated with high frequency of factor V Leiden mutation". Fertil Steril, 74:987-991.**

91. **Younis JS, Brenner B, Ohel G, Tal J, Lanir N, Ben-Ami M. (2000)."Activated protein C resistance and factor V Leiden mutation can be associated with first- as well as second-trimester recurrent pregnancy loss". Am J Reprod Immunol. 43:31-35.**

The web sites references:

1) **http:// www.genome.gov**
2) **5.http://www.ghr.nlm.nih.gov/dynamicImages/chromomap/f5.jpeg)**
3) **http://www.pregnancy on line**
4) **http://www.stago.com**
5) **http://web.indstate.edu/thcme/mwking/blood-coagulation.htm#control**
6) **http://www.ncbi.nlm.nih.gov/sites/entrez**

Appendices

IN The name of the Merciful God
Questionaire

The Relationship between Factor V Leiden Mutation and poor pregnancy outcomes In The West Bank

Health Center --- ---------- --.

File number ---------- ----------.

Date of Birth ----------.

Age at marriage ----------.

Relationship with the husband: ----------.

Height----------

Weight ----------

The degree of education:

1 - Primary 2 -secondary 3- higher than that

Profession:

1 - Housewife 2 Staff member 3- Especial work

Age at first pregnancy (----------).

Result of first pregnancy:

1– Normal delivery 2- Caesarean delivery 3- Abortion

The number of completed pregnancies (_____)
The number of cases of incomplete pregnancy (_____).

- The number of cases of non-completion in the first trimester of pregnancy (_____).

- The number of cases of non-completion of the second trimester of pregnancy (_____).

- The number of cases of non-completion of pregnancy in the third trimester (_____).

Put signal (X) in the right place:

	Question	Yes	NO	I don't recall
1	**Do you have diabetes?**			
2	**Have you suffered earlier from pregnancy diabetes?**			
3	**Do you suffer from high blood pressure?**			
4	**Have you ever suffered from pregnancy toxemia?**			
5	**Do you have varicose legs?**			
6	**Have you suffered earlier from clots in any of your legs?**			
7	**Do you have fiber uterus?**			
8	**Did you use the oral contraceptive pill?**			
9	**Are you a smoker?**			
10	**Have you ever had premature babies?**			
11	**Have you ever a baby born below the weight of 2500 grams?**			
12	**Do you already suffer the death of the fetus before birth?**			
13	**Do you already suffer the death of the fetus during childbirth?**			

Has any one of your relatives (mother or sister) suffered from any of the following diseases?

	Question	Yes	NO	I don't recall
1	Heart infarction.			
2	Lung clots.			
3	Intravenous clots.			
4	Premature delivery.			
5	Babies born below the weight of 2500 grams?			
6	Recurrent abortion.			
7	Placental abruption.			
8	Death of the fetus before birth?			
9	Death of the fetus during childbirth?			

Thank you for the good cooperation

Arabic > English **Error! Hyperlink reference not valid.**

Translate

بسم الله الرحمن الرحيم

" استبانه"

علاقة العامل الوراثي ليدن مع بعض أمراض الحمل عند النساء الفلسطينيات / الضفة الغربية.

المركز الصحي- ---------- رقم الملف---------- الرقم المتسلسل----------

تاريخ الميلاد----------. العمر عند الزواج----------.

صلة القرابة مع الزوج:----------.

الوزن ----------. الطول----------.---

درجة التعليم:

١- ابتدائي ٢- إعدادي ٣- ثانوي ٤- أعلى من ذلك

المهنة:

١ - ربة بيت ٢- موظفة ٣- أعمال خاصة

العمر عند الحمل الأول(----------).

نتيجة الحمل الأول:

١- ولادة طبيعية ٢- ولادة قيصرية ٣- إجهاض

عدد الحمول(_____) عدد الحمول المكتملة (_____)

عدد حالات عدم اكتمال الحمل(_____).

- عدد حالات عدم اكتمال الحمل في الثلث الاول (_____)

- عدد حالات عدم اكتمال الحمل في الثلث الثاني (_____).

- عدد حالات عدم اكتمال الحمل في الثلث الثالث (_____).

ضعي إشارة (X) في المكان المناسب :

	السؤال	نعم	لا	لا اذكر
١	هل تعانين من السكري؟			
٢	هل عانيت من مرض سكري الحمل سابقا؟			
٣	هل تعانين من ارتفاع ضغط الدم؟			
٤	هل عانيت من تسمم الحمل؟			
٥	هل تعانين من دوالي الساقين؟			
٦	هل عانيت من تجلطات سابقة في إحدى ساقيك؟			
٧	هل تعانين من ألياف الرحم؟			
٨	هل استخدمت حبوب منع الحمل؟			
٩	هل أنت مدخنة؟			
١٠	هل سبق وان ولدت ولادة مبكرة؟			
١١	هل سبق وان ولدت أطفال دون وزن ٢٥٠٠ غرام؟			
١٢	هل سبق وان عانيت من وفاة الجنين قبل الولادة؟			
١٣	هل سبق وان عانيت من وفاة الجنين أثناء الولادة ؟			

هل يعاني احد من أقربائك من أي من الأمراض التالية؟

	السؤال	نعم	لا	لا اذكر
١	تجلطات قلبية؟			
٢	تجلطات رئوية؟			
٣	تجلطات وريدية؟			
٤	هل عانت والدتك أو شقيقة لك من ولادة مبكرة؟			
٥	هل أنجبت والدتك أو شقيقة لك أطفال دون ٢٥٠٠ غرام؟			
٦	هل عانت والدتك أو شقيقة لك من اجهاضات متكررة؟			
٧	هل عانت والدتك أو شقيقة لك من تسمم الحمل؟			
٨	هل عانت والدتك أو شقيقة لك من انفصال المشيمة الحاد؟			
٩	هل عانت والدتك أو شقيقة لك من وفاة الجنين قبل الولادة؟			
١٠	هل عانت والدتك أو شقيقة لك من وفاة الجنين أثناء الولادة؟			

وشكرا لحسن التعاون

علاقة الطفرة الوراثية " معامل لايدن " بالإجهاض المتكرر لدى النساء الفلسطينيات في مناطق الضفة الغربية في فلسطين.

إعداد الطالبة:علا داود أبو هلال

إشراف: بروفيسور هشام درويش

ملخص

لوحظ في الآونة الأخيرة ارتفاع نسبة الاجهاضات المتكررة لدى النساء الحوامل في المجتمع الفلسطيني في الضفة الغربية. ولأسباب غير محددة لذا كان الهدف الرئيسي من الدراسة هو تبيان العلاقة بين طفرة العامل الخامس لايدن و الإجهاض المتكرر من أجل تسليط الضوء على هذه المشكلة الصحية الرئيسية ولمعرفة أفضل الحلول للقضاء عليها في المجتمع الفلسطيني.

تم وضع استبيان خاص بموضوع البحث وتوزيعه على النساء المشاركات بالتعاون مع ثلاث مراكز طبية في مدن رام الله وبيت لحم وأريحا وتم اختيار مئة وأربعة نساء فقط ممن انطبقت عليهن شروط الدراسة.وضمت الدراسة المجموعة التجريبية وتشمل ٥٤ من النساء اللواتي يتمتعن بضغط دم طبيعي، وتاريخ مرضي من فقدان الحمل المتكرر (ثلاثة أو أكثر من حالات الإجهاض لأسباب غير معروفة).والمجموعة الضابطة والتي شملت ٥٠ امرأة اللواتي أنجبن على الأقل مرتين دون أي مشكلات صحية تتعلق بالحمل ويتمتعن بشكل عام بصحة جيدة. وقد تم إجراء البحث في مركز البحوث الطبية في جامعة القدس في أبو ديس في الفترة الزمنية (٢٠٠٧-٢٠٠٨) حيث قمنا بعزل الحمض النووي أل DNA من عينا ت دم المشاركات لفحص طفرة العامل الخامس لتخثر الدم لايدن (FVL) باستخدام طريقة التفاعل السلسلي (PCR) وطريقة ARMS Testلفحص المورث (الجين) الخاص بالعامل الخامس للتخثر.

وقد كانت النسبة المئوية للإجهاض المتكرر بين المشاركات في المجموعة التجريبية ٦٨% أما المجموعة الضابطة فقد انتهت جميع حالات الحمل فيها بنجاح وكانت نتائج التوزيع الجيني للعامل الخامس لايدن كالأتي :في المجموعة التجريبية ٢٧,٨% يحملن جين الطفرة (٢٤,١ **%**متغايرة الجينات، ٣,٧ % متماثل الجينات للطفرة) بينما في المجموعة الضابطة ٢٢ % يحملن الصفة متغايرة الجينات للطفرة٠ ولا يوجد تماثل لجينات الطفرة في هذه المجموعة **(P value 0.324)**

مما يدل على انه لا توجد علاقة إحصائية قوية بين " معامل لايدن " والإجهاض المتكرر لدى النساء الفلسطينيات .

كما تم أيضا دراسة تأثير السمنة باستخدام مؤشر كتلة الجسم BMI ولوحظ أنها كانت أعلى قليلا بين النساء في المجموعة التجريبية(٦٦ %) منها في المجموعة الضابطة (٥٧ %) (P value) 0.143كما أن معدل انتشار استخدام OCPs كان أعلى قليلا في المجموعة التجريبية (١٢,٩ %) عنها في المجموعة الضابطة (٨ %) ،**(P value 0.411)** وقد وجد أن نسبة ١٤ % من المستخدمات لأقراص منع الحمل في المجموعة التجريبية يمتلكن الجينات المتغايرة للعامل الخامس لايدن في حين أن جميع المستخدمات لأقراص منع الحمل في المجموعة الضابطة كن يمتلكن جينات طبيعية.

أما بالنسبة لوفاة الأجنة داخل الرحم (IUFD) كانت نسبة حدوثها (بين المشاركات ١١,١ % كلها طبيعية الجين للعامل الوراثي الخامس لايدن. وكذلك لم يلاحظ ارتباط كبير بين هذه الطفرة وتخلف نمو الجنين داخل الرحم IUGR .وقد كانت نسبة توزيع جين الطفرة بين النساء المصابات بإجهاض الثلث الأول من الحمل (٢٨,٣%= ٢٤,٥% متغاير الجينات + ٣,٧% متماثل الجينات للطفرة)أعلى منها بين النساء المصابات بإجهاض الثلث الثاني من الحمل حيث عانت منه امرأة واحدة كانت تحمل الجين بالصورة الطبيعية.

مما سبق نستنتج أن هذه الدراسة لم تستطع إثبات العلاقة بين الطفرة الوراثية " معامل لايدن " والاجهاضات المتكررة لدي النساء في المجتمع الفلسطيني مما يؤكد ضرورة عمل فحوصات مستقبلية على الحمض النووي لجينات عوامل تخثر الدم الأخرى مثل (anticoagulant antithrombin protein C, protein S ، thrombin receptor ..... الخ)على أن تشمل هذه الدراسات الآباء لمعرفة الطرز الجينية المتوقعة للأبناء مع الحرص على متابعة الحالات التي تم استثنائها من الدراسة الحالية .

لذا نوصي بتركيز الجهود على زيادة الوعي الصحي العام لدى أفراد شعبنا حول هذا المرض وعلى عمل الفحوصات اللازمة للأجنة المجهضة لمعرفة أسباب الإجهاض المتكرر وتسجيل حالات الإجهاض في السجل المرضي للنساء بدقة لتوفير ما يلزم من الرعاية الطبية ، بما في ذلك التشخيص المبكر والعلاج الطبي والمواساة لتحسين نوعية حياة الأشخاص المصابين بهذا المرض.