

# Chapter One

## 1. Introduction and literature review:

### 1.1 Recurrent Miscarriage:

Recurrent miscarriage is defined by two or more failed clinical pregnancies and about 50% of reason of recurrent abortion is not clear. Since its original description almost twenty years ago, the antiphospholipid syndrome (APS) has emerged as the most important treatable cause of recurrent miscarriage. The APS is also an important cause of early onset pre-eclampsia and of intra-uterine growth restriction (IUGR) (Bates, 2011).

Approximately 15% of women with recurrent miscarriage have persistently positive tests for either LA or ACA, compared to 2% of those with an uncomplicated obstetric history. In our experience, there is little cross over between LA and ACA positivity and hence It is important to screen for both LA and for IgG and IgM ACA, (Wilson; *et al*, 2008).

Substances in the blood, called phospholipids, are required for the blood to clot. In some people, the body mistakenly identifies phospholipids, or proteins bound to the phospholipids, as foreign substances and forms antibodies against them. This reaction can be viewed as a confusion of the immune system, called an autoimmune process. These antibodies are called Antiphospholipid antibodies. Their presence can lead to blood clots and /or pregnancy loss. However, in some people, they do not cause any problems. Only if a person has had a blood clot or pregnancy loss and test for APLAs that has been positive more than once, measured at least 6 weeks apart, does one refer to the person as having the antiphospholipid antibody syndrome,(Rai; *et al*, 2010).

There are many subgroups of APLAs .the two important are anticardiolipin antibodies (ACA) and lupus anticoagulant. The presence of these antibodies leads to an increased risk of clots and /or pregnancy loss,(Takakuwa; *et al*, 2007).

The majorities of miscarriages are sporadic and most result from genetic causes that are greatly influenced by maternal age (Alaa Eldeen; *et al*, 2013)

The antiphospholipid antibody syndrome is an autoimmune phenomenon. The immune system's function is to watch for and defend against foreign substances in the human body (for instance, bacteria or viruses). One component of this defense system is the antibody. An antibody is a protein that can recognize and bind to a foreign substance,(Takakuwa;*etal*,2007).

Once it has bound to this substance, it can attract other molecules and cells to destroy the offending molecule, (Silver;*etal*, 2009).

In the antiphospholipid antibody syndrome, the body produces antibodies that recognize various molecules in the body that, under normal circumstances, it would not. These molecules (phospholipids for example) play a role in the coagulation cascade along with other functions. The exact mechanism by which the antiphospholipid antibodies and anticardiolipin antibodies induce thrombophilic state is not known, (Silver; *etal*, 2009).

The role of the antiphospholipid antibody syndrome in both arterial and venous thrombotic disorder.

A risk of recurrent thrombi, both arterial and venous, is associated with the antiphospholipid antibody syndrome as well. Most studies suggest that patients who have a recurrent episode will have it in a similar blood vessel type. In other words, patients who have a stroke initially will most often have a stroke if they have a recurrence. None-the-less, patients are reported that have multiple different types of thrombotic events.(Silver;*etal*, 2009).

The antiphospholipid antibody syndrome is also associated with miscarriages as well as other complications of pregnancy including preterm labor and preeclampsia. An association with thrombocytopenia (low platelets) has also been established. This occurs in 20-40% of patients with the antiphospholipid antibody syndrome, (Van den Boogard;*etal*, 2010).

### **1.2 cause of recurrent miscarriages include:**

Genetic,anatomic,endocrine,immunologic,infectious,thrombotic, environmental and unexplained etiologies.

#### **Genetic etiology:**

Approximately 2% to 4% of RM is associated with a parental balanced Structural chromosome rearrangement, most commonly balanced reciprocal or Robertsonian translocations. Additional structural abnormalities associated with RM include chromosomal inversions, insertions, and mosaicism, (Stirrat; 2009).

Single gene defects, such as those associated with cystic fibrosis or Sick cell anemia, are seldom associated with RM.

About 60% of early pregnancy losses associated with sporadic chromosomal anomalies, the risk of sporadic miscarriage between 6 and 12 weeks of gestation in women less than 35 years of age 9% to 12%, the risk increases in women over 35 years of age due to markedly increased incidence of trisomic pregnancies, in women older than 40 years of age the sporadic miscarriage increase to 50%. (Stirrat; 2009).

In the evaluation of recurrent abortion parent should undergo peripheral karyotyping to detect any balanced structural chromosomal abnormalities.

If one of the parents has structural genetic abnormalities pre implantation genetic Testing (PGT) should be done to detect the genetic abnormality of the offspring. Treatment includes pre implantation genetic diagnosis (PGD) for specific translocation, with transfer of unaffected embryos.

IVF/PGD (invitro fertilization) improves live birth rate and structural genetic abnormality, ( Franssen;*et al*, 2011).

**Anatomic Etiologies:**

Congenital uterine abnormalities are associated with second trimester pregnancy loss in addition to other complications, including preterm labor, fetal malpresentation and increased rates of cesarean delivery. Any defect in uterus can be detected by either MRI or 3-D ultrasound imaging. (Christian; *etal*, 2005).

The defect in uterine include unicornuate, didelphic, bicornuate and septate or acute uteri. These anomalies are often detected by MRI or 3-D ultrasound as mentioned above. Septate uterus correct by hysteroscopic surgical and no surgicall corrective option for the didelphic or unicornuate uterus, (Stephenson;*etal*, 2007).

The clinical manegment of pregnancy loss in patient with uterine fibroids and uterine polyps is also controversial, and there is no evidence that surgical treatment reduces the risk of pregnancy loss, (Stephenson;*et al*, 2007).

Anatomic abnormalities account for 10% to 15% of cases of RM and are generally thought to cause miscarriage by interrupting the vasculature of the endometrium, prompting abnormal and inadequate placentation. Thus, those abnormalities that might interrupt the vascular supply of the endometrium are thought to be potential causes of RPL. These include congenital uterine anomalies, intrauterine adhesions, and uterine fibroids or polyps. (RCOG, 2012).

Although more readily associated with second trimester losses or preterm labor, congenital uterine anomalies also play a part in RM. The uterine septum is the congenital uterine anomaly most closely linked to RM, with as much as a 76% risk of spontaneous pregnancy loss among affected patients, (Stephenson, 2012).

**Endocrine Etiologies:**

Luteal phase defect (LPD), polycystic ovarian syndrome (PCOS), diabetes mellitus, thyroid disease, and hyperprolactinemia are among the endocrinologic disorders implicated in approximately 17% to 20% of RM. Traditionally, LPD has been proposed to result from inadequate production of progesterone by the corpus. (Rochet; *et al*, 2008)

Luteal and endometrial maturation insufficient for proper placentation. It is diagnosed when there is a persistent lag of longer than 2 days in the

histologic development of the endometrium compared with the day of the menstrual cycle. Today, the true role of LPD in RM is controversial and endometrial biopsies for LPD diagnosis are rarely indicated. Previous Studies have found evidence of PCOS in at least 40% of women with RM, (Jaslow;*etal*, 2011).

Insulin resistance and the resultant hyperinsulinemia that is often present in cases of PCOS (as well as type II diabetes mellitus) may also play a role in RM , as evidenced by the decreased rate of spontaneous pregnancy loss when patients undergo therapy with the insulin sensitizing drug, metformin. Poorly controlled type 1 diabetes mellitus is also associated with an increased risk of spontaneous abortion, (Rai; *etal*, 2010).

Although untreated hypothyroidism is clearly associated with spontaneous miscarriage, the connection between antithyroid antibodies and RM in euthyroid patients is currently under great debate.(Pental; *et al*, 2010)

There are data to suggest that euthyroid women with antithyroid antibodies, especially those undergoing fertility therapies, are likely to become clinically hypothyroid very soon after the onset of pregnancy, (Stephenson;*etal*, 2007).

Because pregnancy outcomes in these women may improve with early (possibly prenatal) thyroid hormone replacement, similar approaches are presently being studied among women with RPL, (Stephenson;*etal*,2007).

Evaluation of endocrine disorders should include measurement of the thyroid-stimulating hormone (TSH) level. Other testing that might be indicated based on the patient's presentation include insulin resistance testing, ovarian reserve testing, serum prolactin in the presence of irregular menses, antithyroid antibody testing, and, very rarely, luteal phase endometrial biopsies. Therapy with insulin-sensitizing agents for the treatment of RM that occurs in the presence of PCOS has recently gained popularity (Stephenson; *etal*, 2007).

### **Infectious Etiologies:**

Certain infections, including *Listeria monocytogenes*, *Toxoplasma gondii*, rubella, herpes simplex virus (HSV), measles, cytomegalovirus, and coxsackie viruses, are known or suspected to play a role in sporadic spontaneous pregnancy loss. However, the role of infectious agents in recurrent loss is less clear, with a proposed incidence of 0.5% to 5%. The

proposed mechanisms for infectious causes of pregnancy loss include: (1) direct infection of the uterus, fetus, or placenta, (2) placental insufficiency, (3) chronic endometritis or endocervicitis, (4) amnionitis, or (5) infected intrauterine device. Because most of these are isolated events, it appears that there is a limited role for infections as a causative factor in RM. Those particular infections speculated to play a role in RM include mycoplasma, ureaplasma, *Chlamydia trachomatis*, *Leimonocytogenes*, and HSV. The most pertinent risk for RM secondary to infection is chronic infection in an immune compromised patient (Franssen; *et al*, 2011).

Evaluation and therapy should be tailored to individual cases. If a patient with RM has a condition that leaves her immune compromised or a history suggestive of sexually transmitted diseases, evaluation for chronic infections may be warranted. There is no evidence that routine infectious evaluation is appropriate or productive. Immunologic Etiologies because a fetus is not genetically identical to its mother, it is reasonable to infer that there are immunologic events that must occur to allow the mother to carry the fetus throughout gestation without rejection. In fact, there have been at least 10 such mechanisms proposed. It therefore follows that there may be abnormalities within these immunologic mechanisms that could lead to both sporadic and recurrent pregnancy loss, (Christian; *etal*, 2005).

#### **Thrombotic Etiologies:**

Both inherited and combined inherited/ acquired thrombophilias are common, with more than 15% of the white population carrying an inherited thrombophilic mutation. The most common of these are the factor V Leiden mutation, mutation in the promoter region of the prothrombin gene, these common mutations are associated with mild thrombotic risks, and it remains controversial whether homozygous mutations are associated with vascular disease at all. In contrast, more severe thrombophilic deficiencies, such as those of antithrombin and protein S, are much less common in the general population. (Velayuthaprabhu; *et al*, 2005) The potential association between RM and heritable thrombophilias is based on the theory that impaired placental development and function secondary to venous and/or arterial thrombosis could lead to miscarriage (Sierra; *et al*, 2006).

#### **Environmental, lifestyle, Occupational Factors:**

Because of its propensity to result in feelings of responsibility and guilt,

patients are often particularly concerned about the possibility that environmental exposures may have caused their pregnancy losses such as smoking, obesity has been also associated with recurrent pregnancy loss also their life style habits such as cocaine use, alcohol consumption and increase caffeine consumption (>3 cups of coffee have been associated with risk of miscarriage). (Zolghadri, 2004). Links between sporadic and/or recurrent pregnancy losses and occupational and environmental exposures to organic solvents, medications, ionizing radiation, and toxins have been suggested, (Vissenberg, *et al*, 2011).

#### **Male Factors:**

Standard semen parameters, including sperm morphology, do not appear to be predictive of recurrent abortions (Rubio; *et al*, 2011) sperm aneuploidy and DNA fragmentation have a relation with pregnancy loss, abnormal DNA fragmentation may be seen in setting of advanced paternal age or may result from correctable environmental factors such as exogenous heat, toxic exposures, or increased reactive oxygen species in semen(RCOG, 2012).

#### **Unexplained Etiologies:**

Almost half of patients will remain without a definitive diagnosis. The optimal management of these patients is often as unclear as the etiology of their recurrent pregnancy loss. Progesterone has been shown to be beneficial in decreasing the miscarriage rate among women who have experienced at least 3 losses. (Ahmed; *et al*, 2013). Use prior to and during pregnancy has only been proven to increase live birth rates among those women with previous miscarriages beyond 13 weeks of gestation. In fact, the most effective therapy for patients with unexplained RM is often the most simple: antenatal counseling and psychological support. These measures have been shown to have subsequent pregnancy success rates of 86% when compared with success rates of 33% in women provided with no additional antenatal care (Rand, 2004).

#### **1.3 Antiphospholipid Antibodies:**

Antiphospholipid antibodies (APA) comprise a heterogeneous group of autoantibodies directed against negatively charged phospholipids and include lupus anticoagulant (LA) and anticardiolipin antibodies (ACA). (Petri; *et al*, 1998). The importance of these antibodies stems from their established association with thrombosis, thrombocytopenia and recurrent

fetal loss. The first well documented association between antiphospholipid antibodies and recurrent fetal loss was reported in 1975 by Nilsson and coworkers (Rai; *et al*, 2010).

The antiphospholipid antibody syndrome, also known as Hughes Syndrome, is a disorder characterized by multiple different antibodies (protein produced by the body to fight foreign substance) that are associated with both arterial and venous thrombosis (clots in the arterial and veins) (Empson;*et al*, 2005). There are three primary classes of antibodies associated with the antiphospholipid antibody syndrome anticardiolipin antibodies, the lupus anticoagulant and directed against specific molecules these APA appear to be mainly directed against two particular molecules, bêta-2-glycoprotien (B2GPI, anormal protein found in the blood whose function unknown) and another molecules known as prothrombin (normal blood protein that binds to phospholipids and plays Avery important role in blood clotting) (Thiagarajan; *et al*,2003).

Historically, antiphospholipid antibodies were first noted in patients who had positive tests for syphilis without signs of infection. Subsequently, a clotting disorder was associated with two patients with systemic lupus erythematosus in 1952. In 1957 a link between recurrent pregnancy loss and what is now called the lupus anticoagulant was established. Ultimately, the lupus anticoagulant was further described in 1963 and in 1972 the term lupus anticoagulant was given. In1983, Dr. Graham Hughes described the association between antiphospholipid antibodies and arterial as well as venous thrombosis, (Silver; *et al*, 2010).

In 1980 provided further understanding of APA including testing of anticardiolipin (ACA), (De Jong; *et al*, 2011).

In early 1990 discovered that the ACA were found to act against B2GP1, while the lupus anticoagulant fist found to act against B2GP1, more recently, prothrombin, (Bagger; *et al*, 2011) .

There are two main classifications of the antiphospholipid antibody syndrome. If the patient has an underlying autoimmune disorder, such as systemic lupus erythematosus (SLE), the patient is said to have secondary antiphospholipid antibody syndrome. If the patient has no known underlying autoimmune disorder, it is termed primary antiphospholipid antibody syndrome, (Bernner; *et al*, 2002).



### **1.3.1 Signs and complication associated with APA include:**

#### **Vascular thrombosis**

Is one of the complication of APA, can be clots in vein or artery, clot in veins most commonly as deep vein thrombosis (DVT) in the leg, or pulmonary embolism (PE) in lung (due to pulmonary embolism a clot that typically has dislodged from a vein below the pulmonary veins and lodged in pulmonary vein). Clots in the vein less common in eye, the abdomen, or around brain and liver.(Elhassan, 2007).

#### **Embolism**

The blockage the blood vessel caused by a clot that has moved in the blood stream from the site where it formed to a different place in the body. (Dizon; *et al*, 2005).

#### **Recurrent abortion**

APA present in 10-20% in women with recurrent abortion, women with APA have highly risk for pregnancy loss or abortion after 10 week after pregnancy, also, APA associated with other pregnancy complication including eclampsia, preeclampsia, and placental insufficiency, (Deleze; *et al*, 1999).

#### **Thrombocytopenia.**

##### **Low platelet count.**

#### **Certain skin problems:**

Neurological signs, heart valve disease and certain autoimmune disease have been noted associated with APA, (Abalovich;*etal*, 2005).

APA diagnosed in individual who have more episodes of thrombosis or pregnancy loss and there are two main types for APA tests:

Immunological test like ELISA (enzyme linked immune sorbent assay) and coagulation test based for lupus anticoagulant.

In ELISA is immune assay in which antigen-antibody reaction is used to detect antibodies, in coagulation test detected antibody based on their ability to slow down phospholipid dependent clotting reaction (Abalovich;*etal*, 2007)

Treatment for APA individualized according to person's problem, in general in patient who has APA, and has thrombotic event, a short term course of

heparin (is an anticoagulant which is a type of medication used to prevent blood clot from forming or getting bigger) is following by large term warfarin ( another type of anticoagulant), (Bretolacini; *et al*, 2009).

In women who have history with pregnancy loss given blood thinners with aspirin during pregnancy and injection of heparin or low-molecular weight heparin this treatment increase live birth rate about 80% when comparing with other group of female not taking this treatment, (Harris; *et al*, 2012).

Low dose aspirin may improve pregnancy outcome in women with phospholipid antibody by irreversibly blocking the action of cyclo-oxygenase in platelets thereby inhibiting platelet thromboxane synthesis and preventing thrombosis of the placental vasculature.

Heparin may act to reduce fetal loss by binding to phospholipid antibodies, thereby protecting the trophoblast phospholipids from attack and promoting the successful implantation in early pregnancy, in addition its anticoagulant action. (Coutifaris; *etal*, 2008).

The most common type of miscarriage in women with antiphospholipid occurs in the first trimester after fetal heart activity has become established.

Treatment with aspirin and heparin must be stopped in 34 week of gestation to minimize the duration exposure to both aspirin and heparin. (Haas; *etal*, 2008).

APA can be divided into lupus anticoagulant detected by invitro clotting test and antibodies detected by solid –phase enzyme –linked immune sorbent assay (Elisa) which include anticardiolipin antibody (ACA),(Haas; *et al*, 2008).

#### **1.4 Lupus anticoagulant:**

Is an vitro phenomenon in which there is prolongation of phospholipid-dependent coagulation test that is not due to an inhibitor specific to a coagulation factor. It was originally thought that the lupus anticoagulant phenomenon was due to auto –antibodies against anionic phospholipids interfering with the assembly of the tenase and prothrombinase complexes.

Lupus anticoagulant has stronger association with pregnancy loss than the other antiphospholipid antibodies, while the importance of antiB2GP1 and pregnancy loss is uncertain.

Pregnancy failure may be due to thrombosis in the placental bed, although alternative pathogenic mechanisms may apply, and may explain the

tendency to very early losses prior to placentaion. Antiphospholipid APA appear to have a direct affect on trophoblasts and there is evidence for activation of complement in pregnancy failure in experimental APA and in human. These observations may explain the apparent efficiency of heparin in the prevention of early fetal losses in APA, as heparin has been shown to exert potentially beneficial effects on trophoblast in vitro and to inhibit complement activation.

## **1.5Coagulation profile**

### **1.5.1 Hemostasis**

Hemostasis is the result of several interaction system designed to prevent or stop bleeding. Integral part of the active hemostatic process include platelet activity intrinsic and extrinsic coagulation also known as tissue factor pathway release of vasoconstrictors and vasodilators systemic removal of clots by fibrin lysis countering each of the active processes that must be considered in order to appreciate hemostasis in complete terms.

Control system include release of endothelial prostaglandin to inhibited platelet activation and quiet activated platelets, plasma serine protease inhibitor that neutralize active clotting and lytic factors escaping from the thrombus site, and the protein s and protein c control system that limits clotting by interaction of pivotal co factors V and VIII . the balancing act makes normal clotting and repair possible without consuming all of the haemostatic elements in each event. Bleeding disorders occur when the balance is tipped in one direction and cannot rebound. This may be caused by consumption of elements necessary for hemostasis or genetic deficiency in their production.

### **1.5.2 Primary Hemostasis:**

During primary hemostasis, platelet generally display for district properties:

#### **(1)Platelet adhesion**

Following blood vessel injury, platelet adhere to exposed sub endothelial connective tissue bind platelets by von- well brand factor through GPIIb, GPIIIa become exposed and form secondary binding with von- well brand factor further promoting adhesion. Adhesion to collagen facilitated by GPIa, (Peranettef, 2005).

Platelet adhesion induce series of metabolic reaction which initiate the platelets released reaction, shape change and aggregation. The key response is activation of phospholipase A2 (PLA2) and (PCL) and other enzyme which together stimulate prostaglandin metabolism and phosphoinositide turn over, (Hoffbrand; *etal*, 2006).

**(2) Platelet shape change:**

Within seconds of their adhesion to vessel wall components or, indeed, to non physiological surface such as glass, platelet undergo change in shape to become more spherical and putting out long, spiny pseudopods which form the initial points of contact and enhance interaction between adjacent platelets. The shape change is accompanied by recognition of the internal constitute of the platelets, (Hoffbrand; *etal*, 2006).

**(3) Platelets release reaction:**

Immediately following their adhesion and shape change, platelets commences a specific release reaction which sustained for several minute and the intensity of which varies with stimulus. Weak inducers such as low doses of ADP or adrenaline involve mainly the alpha granule contents aproportion of which may even leak out from un stimulated platelet in circulated blood, Higher concentration of ADP or adrenalin and low dose of collagen result secretion from both alpha granule and dense bodies, while strong stimuli such as thrombin or high dose of collagen, cause release of lysosomal enzyme as well, (Christopher, 2004).

Collagen exposure or thrombin action result in the secretion of platelet granules content which lead to platelet aggregation and enhance the coagulation, (Hoffbrand;*etal*, 2006).

**(4) Platelet aggregation:**

Wide variety of substance binds to platelets, often via specific receptors and there after induce platelet aggregation or agglutination.

Under physiological condition only ADP and TXA2 are likely to reach the concentration required to induce aggregation and then only locally and transiently at point of vascular injury, (Hoffbrand;*etal*, 2006).

ADP cause platelet to swell and encourage the platelet membrane of adjacent platelet to adhere to each other this result in the formation of platelet mass large enough to plug the area of the endothelial injury, (Brenner; *et al*, 2002).

### **1.5.2 Secondary hemostasis:**

It occurs when soluble plasma protein called coagulation factor interact in series of enzymatic reaction to convert the soluble protein (fibrinogen) to insoluble fibrin.

The reaction occurs in coagulation cascade divided into three interacting pathway:

1. The intrinsic pathway.
2. The extrinsic pathway.
3. The common pathway.

#### **(1) Intrinsic pathway:**

The component of its all contained within blood stream, hence the name intrinsic.

It initiated by exposure of contact factors (factor XII, factor XI, HMWK, prekallikrein) to vessel structure beneath the endothelium collagen basement membrane. The contact phase begin within factor XII which activation to serine protease (XIIa) factor XIIa it will activates factor XI to Xia in the present of HMWK as co factor.

Factor XIIa activates prekallikrein to serine protease kallikrein the HMWK also act as cofactor in this reaction. Kallikrein activates additional factor XII to XIIa. Factor Xia activates factor IX which is vitamin K- dependent. This step requires calcium bound to phospholipids surface (PF3) in order for factor IX to attach.

Factor IX a in turn activates factor X which is also vitamin K-dependent factor requiring calcium. Modified factor VIII act as cofactor to hold the two together in proper spatial arrangement for activation of X to Xa. The first component of common pathway factor IXa, VIIIa, calcium, PF3. Known as tenase complex. The intrinsic pathway can evaluate in the activated partial thromboplastin test (APTT), (Peranettef, 2005).

#### **(2)Extrinsic pathway:**

Factor X can also be activated by extrinsic pathway which activation following vascular endothelial cell injury.

The tissue factor (TF) will bind with factor VII to form TF. VII complex, this complex is enzymatic it will activate factor VII to VIIa through factor Xa, IXa and another pathway TF.VIIa complex bind with calcium to activation factor X first component of common pathway. (Besa;*etal*, 2008).

### **(3) Common pathway:**

The common pathway includes major three reactions:

- (a) Activation factor X by extrinsic and intrinsic pathway.
- (b) Factor Xa convert prothrombin (factor II) to thrombin in the presence of factor Va and calcium.

Factor IIa (thrombin) it convert fibrinogen (factor I) to fibrin polymer which stabilizing by factor XIIIa to formation of stable fibrin clot. (Besa; et al, 2004).

© (factor XIII also activated by thrombin in the presence of calcium). (Perandettef, 2005).

### **1.6 Fibrinolysis:**

It's generally acknowledged that the fibrinolytic system plays an important role in removing fibrin from intravascular and extra vascular sites. Moreover, it is becoming increasingly apparent that aberrations of the fibrinolytic response can have catastrophic clinical consequences in terms of both hemorrhagic and thrombotic events.

Despite this, fibrinolysis has remained the poor relation of hemostasis in general, with only an occasional attempt being made by clinicians to interfere with the course of nature and few laboratories willing to undertake any but the simplest tests. (Tagwa; *et al*, 2013).

e.g fibrinogen degradation product (FDP) assay. The two main reasons for these shortcomings have been firstly, the difficulty in reconciling the results of time consuming laboratory tests with the rapidly changing clinical condition of the patient, and, secondly, the lack of safe, effective therapeutic materials with which to treat fibrinolytic disorders. However, simple, rapid, assays for components of the fibrinolytic system, based on chromogenic substrate, have now been developed and synthetic t-PA produced by recombinant DNA technology has recently become available together with clarification of the physiological and pathological mechanisms involved in fibrin degradation, these advances have enabled clinicians to treat fibrinolytic disorders more effectively, less empirically and with more comprehensive laboratory support than was hitherto the case.

### **1.6.1 Component of the fibrinolytic system:**

These include plasminogen, plasminogen activators and anti plasmins, as well as the products of fibrin degradation. Although enzymes derived from leucocytes (e.g elastase) contribute in minor way to clot lysis by far the most important route is fibrin degradation induced by plasmin the inactive precursor of which is plasminogen.

### **1.7 Treatment:**

The treatment recommendations for patients with recurrent miscarriage are based on the causes of recurrent miscarriage. The low molecular weight of heparin and aspirin is prescribed for women with unexplained recurrent miscarriage which is standard treatment based on hypothesis that may condition might be caused by thrombosis in decidual vessels.

The treatment with heparin and aspirin increase rate of live birth 75\_80% (Ahmed; *et al*, 2013).

## **1.8 Rationale:**

Recurrent miscarriage is a critical problem in which many factors play a role in it such as Antiphospholipid antibodies (APA).

Recent study pointed to potential role of antiphospholipid antibodies of possible cause of recurrent miscarriage.

In our center we found that among 60 patients whom 21 suffering from recurrent miscarriages for it choose the topic.

Previous Indian studies showed abnormal values of PT and APTT in females with recurrent abortion.

This research may help to investigate and analyze the effect of antiphospholipid antibodies on recurrent miscarriage.



## **1.9 Objectives:**

### **1.9.1 General objective:**

To determine prevalence of antiphospholipid antibodies in Sudanese women with recurrent miscarriage.

### **1.9.2 Specific objectives:**

- 1- To measure coagulation parameters prothrombin time and activated partial thromboplastin time in women with recurrent miscarriages.
- 2- To determine antiphospholipid prevalence in women with recurrent miscarriage .
- 3-To determine lupus anticoagulant prevalence in women with recurrent miscarriage.
- 4-To compare these parameters in females at different age group.
- 5- To comparison between women who taking treatment (heparin and aspirin) and those who did not taking treatment.

## **CHAPTER TWO**

### **Material and Methods:**

#### **2.1 study design:**

This descriptive and analytical study conducted in Dr. Elsir Fertility Center (EFC) in Khartoum state in period from May 2013 to March 2014 study antiphospholipid antibody and prothrombin time and activated partial thromboplastin time among Sudanese women with recurrent miscarriage.

#### **2.2 Study population:**

This study conducted based on a number of 100 Sudanese women suffering from miscarriage with an age ranging from 20\_40 years old, a number of 50 samples from normal non pregnant females as control group.

All participants interviewed by special questionnaire used to investigate several parameters among all participants.

#### **2.3 Inclusion and exclusion criteria**

The inclusion criteria included that the women with history with recurrent miscarriage with unknown cause of recurrent miscarriage and were not on any anticoagulant pills, and any patient with known cause with miscarriage was excluded from this study.

#### **2.4 Sample size:**

100 blood samples were taken from patients suffering from recurrent miscarriages, and 50 blood samples were collected from healthy female.

#### **2.5 Sampling:**

Five ml of venous blood were obtained from each patient and control by clean venipuncture, and divided between plain tube (for APA) and trisodium citrate for PT and APTT. Amount of 1.8 ml of venous blood added in Tri Sodium Citrate as anticoagulant containers and then mixed gently. Then the sample centrifugated at 2500 rpm for 15 minute. And other 3.0 ml of venous blood collected in plain container the serum used for APA by using Elisa.

#### **2.6 Materials:**

Thromboplastin, Ca for PT.

Kaolin and cephalin +  $\text{CaCl}_2$  for APTT.

Biobas 10 model CD500.98.

Elisa machine and reader (humareader) for APA.

Orgentec kits for APA.

## **2.7 Methods:**

### **2.7.1 Antiphospholipid antibody:**

#### **Principle:**

A mixture of highly purified cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and human beta-2-Glycoprotein I is bound to microwells.

Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end product. The intensity of the yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration present in the original sample.

#### **Procedure:**

Serum sample from each case and control was tested for APA. 100  $\mu\text{l}$  of calibrator controls and prediluted patient samples was pipette into the wells. Then Incubated for 30 minute at room temperature. The contents of the micro wells were discarding and washed 3 times with 300 $\mu\text{l}$  of wash solution after that 100  $\mu\text{l}$  of enzyme conjugate was added to each well, incubated for 15 minute at room temperature. after that discard the contents of microwells and washed 3 times with 300  $\mu\text{l}$  of wash solution, 100  $\mu\text{l}$  of TMB substrate was added, incubated for 15 minute at room temperature and 100 $\mu\text{l}$  of stop solution was added and incubated for 5 minute then optical density was read at 450 nm and result was calculated.

### **2.7.2 Prothrombin time (PT):**

Uses: To evaluate the extrinsic pathway.

**Principle:**

The test measures the clotting time of plasma in the presence of optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. Although originally thought to measure prothrombin, the test is now known to depend also on reaction with factors V, VII, X and on the fibrinogen concentration of the plasma (Dacie, 2006).

**Procedure:**

Plasma sample from each case was tested for Prothrombin time (PT), 100µl of control and patient citrated platelet poor plasma pipette into a warmed cuvette. Then, 200µl of calcified thromboplastin were added, test was performed in duplicate. End point was observed and the mean of the double determination was plotted.

**Normal values:**

Normal values depend on the thromboplastin used; with most rabbit thromboplastins, the normal range of the PT are between (11\_16) second.

**2.6.3 Activated partial thromboplastin time (APTT):**

This test is also known as the partial thromboplastin time with kaolin (PTTK) and the coagulation cephalin clotting time (KCCT) reflecting the method used to perform the test.

Uses: to evaluate the intrinsic pathway.

**Principle:**

The test measure clotting time of plasma after activation of the contact factor but without added tissue thromboplastin, and so indicates the overall efficiency of the intrinsic pathway. To standardize the activation of the contact factor, the plasma is first pre-incubated with kaolin or elagic acid. Standardized phospholipids are provided to allow the test to be performed on platelet poor plasma. The test depends not only on the contact factors and on factor VIII and IX but also on the reactions with factors X, V, prothrombin and fibrinogen. It is also sensitive to the presence of the circulating anticoagulants (inhibitors) and heparin, (Dacie, 2006).

**Procedure:**

Plasma sample from each case and control was tested for APTT, 100 $\mu$ l of control and patient citrated platelet poor plasma were added into a warmed cuvette , and then 100 $\mu$ l of cephalin-Koalin mixture was added and incubated for 2 minute. Following that, 100 $\mu$  l of  $\text{CaCl}_2$  was added and the end point was observed. The mean of the double determination was plotted.

**Normal values:**

Normal values (20-40) second.

**2.7.4 APTT mixing with normal plasma:**

By mixing patients plasma with normal pooled plasma, factor deficiency can be differentiated from inhibitors. An inhibitor, if present, can generally characterize as lupus- like.

Procedure of APTT with normal plasma 50ml of patient citrated plasma were added to 50ml of normal citrated platelet poor plasma and same dilution for control plasma and same procedure of APTT were followed with it.

**2.8 Data analysis:**

The collected data coded in master sheet and proceed for analysis using SPSS version 20s computerized program. Independent T test and Chi2 were used.

**2.9 Ethical consideration:**

An informed consent was obtained from each volunteer. The confidentiality of the patients was established by coding of the questionnaires and the data list by a different code from their files to insure the anonymity of respondents. All investigations were carried out for patients free of charge.

## Chapter three

### Result

**Table (1)**

Age distribution among patients with recurrent abortion and control group

Age group	Patient	Control
<b>20-24</b>	2(2.3)	12(24.0)
<b>25-29</b>	19(21.6)	20(40.0)
<b>30-34</b>	32(36.4)	18(36.0)
<b>≥35</b>	35(39.8)	0
<b>Total</b>	88	50

**Table (2):**

Average distribution of PT, APTT and Antiphospholipid in patient and control

	<b>PT mean± SD</b>	<b>APTT mean± SD</b>	<b>Antiphospholipid mean± SD</b>
<b>Patient</b>	12.7±2.47	34.08±8.16	4.36±6.53
<b>Control</b>	11.99±0.95	29.17±3.27	2.12±1.44
<b>P-value</b>	0.017	0.000	0.003

**Table (3):**

Average distribution of PT, APTT and Antiphospholipid based on number of recurrent miscarriage

<b>Recurrent abortion</b>	<b>PT mean<math>\pm</math> SD</b>	<b>APTT mean<math>\pm</math> SD</b>	<b>Antiphospholipid mean<math>\pm</math> SD</b>
<b>Twice</b>	12.0 $\pm$ 0.95	33.0 $\pm$ 8.95	3.69 $\pm$ 3.84
<b>Three and more</b>	12.81 $\pm$ 2.62	34.25 $\pm$ 8.08	4.46 $\pm$ 6.87
<b>P-value</b>	0.293*	0.625*	0.707*

\*p-value >0.05 is not statistical significant different.

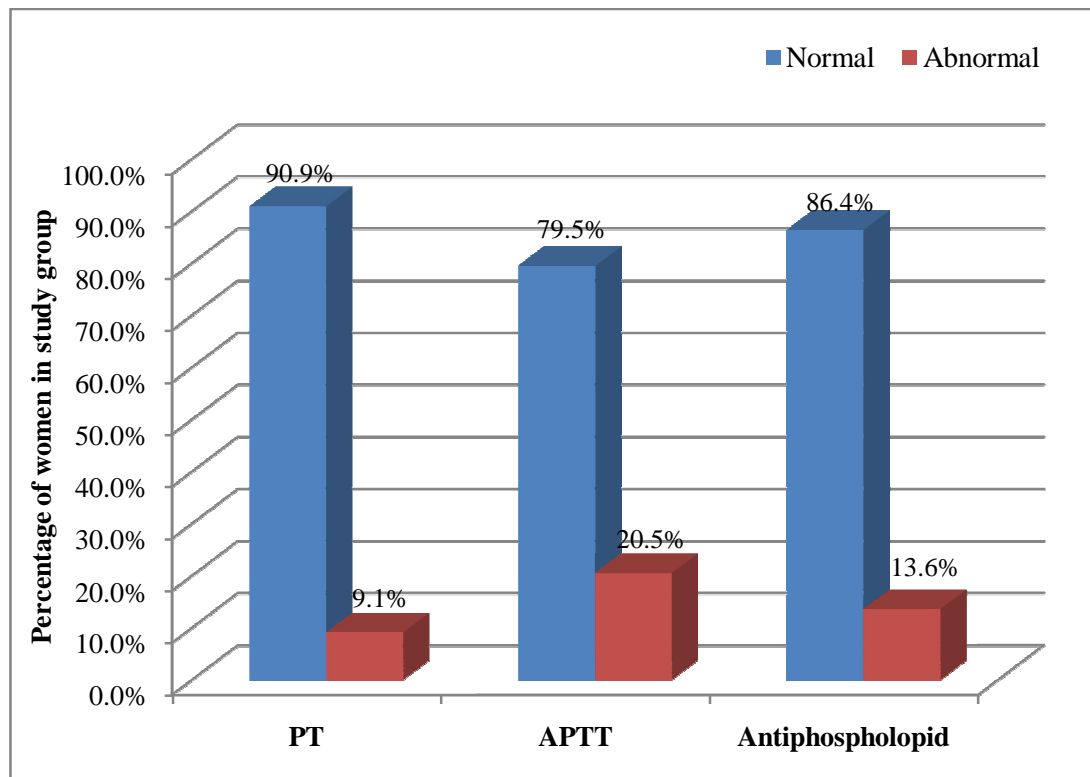


**Table (4):**

Distribution of mean Antiphospholipid based on gestational age of recurrent miscarriage.

<b>Gestational age of abortion</b>	<b>Antiphospholipid</b>	
	<b>Normal N (%)</b>	<b>Abnormal N (%)</b>
<b>≤16 weeks</b>	46(60.5)	10(83.3)
<b>&gt;16 weeks</b>	30(39.5)	2(16.7)

**Figure (1)**



Distribution of PT, APTT and Antiphospholipid in study group

**Table (5):**

Distribution of live birth in recurrent miscarriage women with and without taken Heparin/Aspirin

Heparin/Aspirin	Birth	
	Live N (%)	Dead N (%)
Not taken	4(6.1)	26(76.5)
Taken	62(93.9)	8(23.5)
P-value	0.000	

\*P-value<0.001 is highly statistical significant different

**Figure (2)**

Distribution of patients and controls

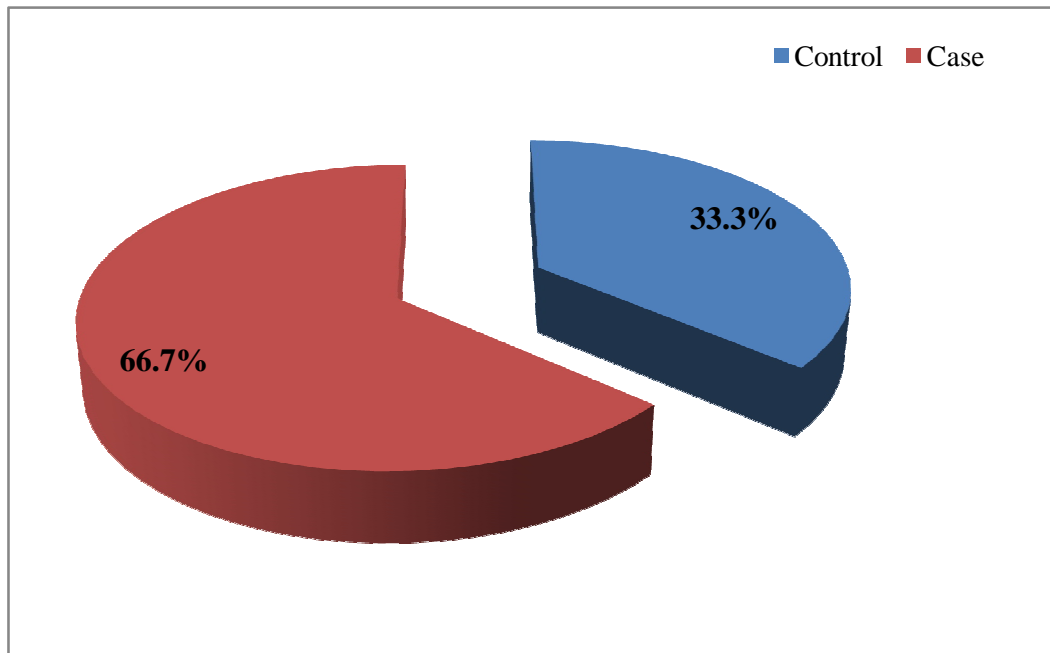


Table (1) shows the distribution of patients and controls.

Table (2) shows the average distribution of PT, APTT and Antiphospholipid Patients and controls.

Table (3) show average distribution of PT, APTT and Antiphospholipid based on number of recurrent miscarriage.

Table (4) show distribution of mean Antiphospholipid based on gestational age of recurrent miscarriage using Chi2 test which found the women with recurrent miscarriage <16 week about 83.3% while the women >16 week were 16.7%.

Figure (1) show distribution of PT, APTT and Antiphospholipid in study group which found 13.6% of women with recurrent miscarriages have APA and 9.1% have prolonged PT and 20.5% have prolonged APTT.

Table (5) show the distribution between recurrent miscarriage women who taking heparin and aspirin and not taking, which found the women who treated by heparin and aspirin increase live birth and P-value 0.000 which is highly statistical significant.

Figure (2) show distribution between case and control.

## **Chapter four**

### **4.1 Discussion**

Repeated abortion occurs in the practice of Obstetrics & Gynecology in this study APA can explain the percent in Sudanese women with recurrent miscarriage and the prothrombin time (PT), activated partial thromboplastin time (APTT) and lupus anticoagulant. Antiphospholipid syndrome characterized by recurrent arterial or venous thromboembolism or pregnancy loss due to antibodies directed against anionic phospholipids. Fetal demise in caused comment by antiphospholipid antibodies is due to infraction of blood vessel of the placenta. This study is added to some previous studies in Sudan which shows statistically significant difference of prothrombin time (PT), activated partial thromboplastin time (APTT) and antiphospholipid antibodies in recurrent miscarriage. Also the data in this study extend and confirm the data obtained in other studies in Sudan carried out by Elhassan and Ahmed (2007) and other countries.

This study shows that women with unsuccessful pregnancies had mounted levels of APA. These agree with the reported frequencies of APA in many previous studies from India, Iran and Oman.

The study was found 13.6% of women with recurrent miscarriage had positive antiphospholipid antibodies and this result was significant and was in line with reported study in Iran carried out by Zolghadri (2007) and India carried out by Velayathrabhu (2009); although its considered a high percentage but it was lowest percentage of APA among Sudanese women with recurrent miscarriage when compared to other studies conducted in Sudan and other countries 20-26%.

In Indian study the frequencies of antiphospholipid antibodies (APA) and lupus anticoagulant in sera of women with recurrent miscarriage were 25% carried by Velayathrabhu (2009).

In Iran the frequencies of antiphospholipid antibodies in sera of women with recurrent miscarriage 19.4% carried out by Zolghadri (2004). Another report by Vaidyanathan and others from Oman shown that the frequencies of antiphospholipid antibodies were 23%.

The data in our study confirm and extend the data obtained by other study by Elhassan (2007), Velayutyaprabhu (2009) and Zolghadri (2007).

According to the result of the current study, 20.5% of women with recurrent miscarriage had prolonged APTT. That was agreement with a previous study in Sudan by Elhassan, (2007).With a significant difference between the frequencies of the two studies.

Also the other result of study in India done by Velayuthaprabhu , (2009), was agree with result, in this study there is significant difference and found 16% of women with recurrent miscarriage had prolonged APTT, Rochat (2008), observed in his study done in Italian women that APTT was prolong and significant agree with silver.

This study also show 9% of women with recurrent miscarriage had prolonged PT that was agree with previous study in Sudan by Elhassan (2007), found 10% of women with recurrent miscarriage had prolonged PT. and other study in Sudan also was found 5% from women with recurrent miscarriage had prolong PT this study done by Ahmed (2013).This result was obtained by study performed in women with recurrent miscarriage in Greece, reverted that prothrombin time was prolonged in 13%, Bretolacini (2009). 20.5% of prolonged APTT 8% was corrected when added 50:50 mixture of patient and normal pooled plasma that mean deficiency in intrinsic factor while 12% failed to correct the prolong activated partial thromboplastin time (APTT) when added 50:50 mixture of patient and normal pooled plasma that is due to the presence of inhibitor in sera obtained from these women mainly lupus anticoagulant. This agree with studies from India by Velayutharbhu (2009) he found 16% LA, also agree with Zolgadri from Iran (2007) which found 18.6% from women with RM have LA.

Another report by Ahmed; (2005) showed that only 10% of women with recurrent miscarriage had prolonged APTT and that due to the presence of inhibitors mainly lupus anticoagulant (LA).

In this study was found there is no clinical significant in PT, APTT and APA according to numbers of recurrent abortion this result was agreed with Elhassan (2007), and antiphospholipid based on number and gestational age of miscarriage.

## **4.2 Conclusion:**

1. The data concluded that the presence of antiphospholipid antibody in sera of women is significantly associated with recurrent miscarriage and the age, and the frequency of secondary antiphospholipid syndrome was higher than the frequency of primary APA.
2. Prolongation in PT may be due to the presence of anti-thrombin antibodies in serum of patients with recurrent miscarriage.
3. The activated partial thromboplastin time (APTT) was more sensitive than prothrombin time (PT) in the presence of lupus anticoagulant.
4. Some women with recurrent miscarriage had prolonged APTT due to deficiency in intrinsic factor or due to inhibitors.
5. In these study women with recurrent miscarriage 13.6% positive for APA
6. Women with APA who treated with Aspirin/heparin had successful pregnancy and had live birth when compared with other women not taking Aspirin/heparin.
7. Heparin/Aspirin is successful treatment for women who had APA but not for women with unexplained recurrent miscarriage.



### **4.3 Recommendations**

1. We recommended that bigger sample size and automated system used to deal with big sample size and to give more reliable results.
2. Other confirmatory test should be done such as (DRVVT) dilute Russell Viper Venoms time because appeared to be the most sensitive test, while the APTT were the most specific for Lupus anticoagulant.
3. Administration of heparin combined with low dose aspirin is recommended, in women with APS.
4. In general treatment should begin as soon as pregnancy confirmed.

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