Chapter one  
Introduction, Rationale, objectives

1.1 Introduction:

Pregnancy loss is secondary to multiple illnesses. The causes of recurrent miscarriage are ranging from genetic factors, hereditary thrombophilia, endocrine and metabolic disorder, uterine abnormalities (Dohont, 2003).

An important cause is the antiphospholipid syndrome, an autoimmune disease with various clinical alterations (Galindo et al, 2007). Antiphospholipid syndrome has received considerable attention from the medical community because of its association with a number of serious clinical disorders (Gezer, 2003). Antiphospholipid antibody syndrome is characterized by autoantibodies against negatively charged phospholipids in the serum, and clinically by multiple thrombosis, thrombocytopenia, and recurrent fetal loss.

The antiphospholipid syndrome was reported in the early 1980s as the association of thrombosis, recurrent pregnancy loss in the presence of anticardiolipin antibodies and/or lupus anticoagulant. Since then, many other clinical manifestations have been associated with antiphospholipid. Almost any organ and tissue may be involved in the disease, including the brain, the heart, the kidneys, the placenta and many more (Cuadrado and Lopez, 2003).

Antiphospholipid syndrome is characterized by recurrent arterial or venous thromboembolism or pregnancy loss in association with antibodies directed against anionic phospholipids or plasma proteins bound to anionic phospholipids. In accordance with this, fetal abortion, typically beyond the tenth week of gestation, is also caused by infarctions of blood vessels in the placenta (Specker, 2007).

Among the autoimmune factors, anti-phospholipid antibodies have been demonstrated to be the strongest risk factors for fetal loss, the prevalence of
which is as high as 40% in women with recurrent fetal loss (Shetty and Ghosh, 2009).

But the pathophysiologic mechanisms that characterize thrombosis and recurrent pregnancy losses are still not clear (Peluso and Morrone, 2007). Thrombotic events at the placental level cannot explain all of the clinical manifestations. It has been suggested that anticardiolipin may be responsible for a local acute inflammatory response mediated by complement activation and neutrophil infiltration eventually leading to fetal loss (Meroni et al, 2010).

Obstetric complications such as fetal death, premature delivery, preeclampsia and recurrent abortions are characteristic manifestations of antiphospholipid syndrome (Levy, 2010).

The antiphospholipid antibody syndrome is an autoimmune condition in which vascular thrombosis and/or recurrent pregnancy losses occur in patients (Rand, 2003).

risk factors for recurrent pregnancy loss and obstetric complications which is characterized by recurrent fetal loss, thrombosis, and thrombocytopenia in association with anticardiolipin antibodies (Velayuthaprabhu and Archunan, 2005).

1.2 Rationale:
Recurrent miscarriage affects 2-5% of population (Wilcox, 1988).

The causes of recurrent miscarriage are multiple; antiphospholipid syndrome (APS) represents significant cause of recurrent miscarriage recent studies pointed to potential role of antiphospholipid antibodies of possible cause recurrent miscarriage.

The risk of recurrent fetal loss is significantly higher in pregnant women with antiphospholipid antibodies or lupus anticoagulant and anticardiolipin, women with anticardiolipin antibodies have 3-9 times greater risk of fetal loss, than those who had not have them, antiphospholipid antibodies facilitate arterial and venous thrombosis.

In Sudan limited data concerning APL among Sudanese pregnant women with recurrent miscarriage, the results of this study could highlight’s the clinical problem of pregnant women and correlates APL with recurrent miscarriage.
1.3 Objectives:

1.3.1 General objectives:
To determine the frequency of antiphospholipid among Sudanese women with recurrent miscarriage.

1.3.2 Specific objectives:
1.3.2.1. To determine the Frequency of women with recurrent miscarriage who are positive test for anticardiolipin antibody

1.3.2.2. To compare between the antiphospholipid, anticardiolipin and (number of miscarriages, and family history).

1.3.2.3. To compare the antiphospholipid and anticardiolipin antibodies in different female age group
Chapter Two

Literature Review

2.1 pregnancy:

Pregnancy, also known as gravidity or gestation, is the time during which one or more offspring develops inside a woman. Pregnancy can occur by sexual intercourse or assisted reproductive technology. It usually last around 40 weeks (10 lunar months) from the last menstrual period (LMP) and ends in childbirth (Abam and Steven, 2011).

This is about 38 weeks after conception. An embryo is the developing offspring during the first 8 weeks following conception, after which, the term fetus is used until birth.

Pregnancy is typically divided into three trimesters. The first trimester is from week one through twelve and includes conception. Conception is followed by the fertilized egg traveling down the fallopian tube and attaching to the inside of the uterus, where it begins to form the fetus and placenta. The first trimester carries the highest risk of miscarriage (natural death of embryo or fetus). The second trimester is from week 13 through 28. Around the middle of the second trimester, movement of the fetus may be felt. The third trimester is from 29 weeks through 40 weeks. (Reynold et al, 2002).

2.2 Miscarriage and Recurrent Miscarriage:

The definition of miscarriage is usually is loss of pregnancy before viability, the WHO definition of miscarriage is fetal death in early pregnancy. still birth is fetal death on late pregnancy. miscarriage can divide in two broad categories sporadic and recurrent miscarriage
Sporadic is miscarriage is the most common complication of pregnancy, occurs in up to 15% of all recognized pregnancies, at least 25% of women with experience of at least one sporadic miscarriage in their reproductive live (Jivraj, 2009).

Recurrent miscarriage is usually define as three or more consecutive spontaneous miscarriage occurring in first trimester with same biological father, they may or may not follow successful birth, about half of recurrent miscarriage are unexplained (Duckitt and Qureshi, 2011).

Some authorities define recurrent miscarriage of two more consecutive pregnancies if this definition is used, 5% of women is affected by recurrent miscarriage, most investigator perform diagnostic evaluation after three miscarriage (Bates, 2011).

2.3 Causes of Recurrent Miscarriage:

There are various causes for recurrent miscarriage, and some are treatable. Some couples never have a cause identified, often after extensive investigations. About 50-75% of cases of Recurrent Miscarriage are unexplained.

2.3.1 Anatomical conditions:

2.3.1.1 Uterine conditions:

A uterine malformation is considered to cause about 15% of recurrent miscarriages. The most common abnormality is a uterine septum, a partition of the uterine cavity. The diagnosis is made by MRI or a combined laparoscopy hysteroscopy of the uterus. Also uterine leiomyomata could result in pregnancy loss (Strirrat, 2009).

2.3.1.2 Cervical conditions:
In the second trimester a weak cervix can become a recurrent problem. Such cervical incompetence leads to premature pregnancy loss resulting in miscarriages or preterm deliveries.

2.3.2 Chromosomal disorders:

2.3.2.1 Translocations:

A balanced translocation or Robertsonian translocation in one of the partners leads to unviable fetuses that are miscarried. This explains why a karyogram is often performed in both partners if a woman has experienced repeated miscarriages. About 3% of the times a chromosomal problem are more likely to miscarriage, they may also deliver normal or abnormal (Strirrat, 2009).

2.3.2.2 Aneuploidy:

Aneuploidy may be a cause of a random spontaneous as well as recurrent pregnancy loss. Aneuploidy is more common with advanced reproductive age reflecting decreased germ cell (Strirrat, 2009).

2.3.3 Endocrine disorders:

Women with hypothyroidism are at increased risk for pregnancy losses. Unrecognized or poorly treated diabetes mellitus leads to increased miscarriages. Women with polycystic ovary syndrome also have higher loss rates possibly related to hyperinsulinemia or excess androgens. Inadequate production of progesterone in the luteal phase may set the stage for RPL (Rodger et al., 2008).

2.3.4 Thrombophilia:

An important example is the possible increased risk of miscarriage in women with thrombophilia (propensity for blood clots). The most common problem is the factor V Leiden and prothrombin G20210A mutation. Some preliminary studies suggest that anticoagulant medication may improve the chances of
carrying pregnancy to term but these studies need to be confirmed before they are adopted in clinical practice (Rodger et al, 2008).

Note that many women with thrombophilia go through one or more pregnancies with no difficulties, while others may have pregnancy complications. Thrombophilia may explain up to 15% of recurrent miscarriages (William, 2012).

2.3.5 **Immune factor:**

A common feature of immune factors in causing recurrent pregnancy loss appears to be a decreased maternal immune tolerance towards the fetus. (William, 2012).

2.3.5.1 **Antiphospholipid syndrome:**

The antiphospholipid syndrome is an autoimmune disease that is a common cause of recurrent pregnancy loss around 15% of the women who have recurrent miscarriages have high levels of antiphospholipid antibodies. Women who have had more than one miscarriage in the first trimester, or a miscarriage in the second trimester, may have their blood tested for antibodies, to determine if they have antiphospholipid syndrome. Women diagnosed with antiphospholipid syndrome generally take aspirin or heparin in subsequent pregnancies, but questions remain due to the lack of high quality trials.(Empson et al, 2005).

2.3.5.2 **Thyroid antibodies:**

Anti-thyroid auto antibodies are associated with an increased risk of recurrent miscarriage with an odds ratio of 2.3 with a 95% confidence interval of 1.5–3.5. (Vanden, 2011).

2.3.5.3 **Increased uterine NK cells:**
A controversial area is the presence of increased natural killer cells in the uterus. It is poorly understood whether these cells actually inhibit the formation of a placenta, and it has been noted that they might be essential for this process, determination of NK cells in peripheral blood does not predict uterine NK cell numbers, because they are a different class of lymphocytes, and state that immunosuppressive treatments are not warranted (Nielsen, 2011).

2.3.5.4 Parental HLA sharing:

Earlier studies that perhaps paternal sharing of HLA genes would be associated with increased pregnancy loss have not been confirmed.

2.3.5.5 Male-specific minor histocompatibility:

Immunization of mothers against male-specific minor histocompatibility (H-Y) antigens has a pathogenic role in many cases of secondary recurrent miscarriage, that is, recurrent miscarriage in pregnancies succeeding a previous ample of this effect is that the male: female ratio of children born prior and subsequent to secondary recurrent miscarriage is 1.49 and 0.76 respectively (Nielsen, 2011).

2.3.6 Ovarian factors:

2.3.6.1 Reduced ovarian reserve:
The risk for miscarriage increases with age, and women in the advanced reproductive age who have a reduced ovarian reserve are prone to higher risk of repeated miscarriages. Such miscarriages are due to decreased egg quality (Vissenberg and Goddijn, 2011).

2.3.6.2 Luteal phase defect:

The issue of a luteal phase defect is complex. The theory behind the concept suggests that an inadequate amount of progesterone is produced by the corpus luteum to maintain the early pregnancy. Assessment of this situation was traditionally carried out by an endometrial biopsy, however recent studies have not confirmed that such assessment is valid. Studies about the value of progesterone supplementation remain deficient; however, such supplementation is commonly carried out on an empirical basis (Vissenberg and Goddijn, 2011).

2.3.7 Lifestyle factors:

While lifestyle factors have been associated with increased risk for miscarriage in general, and are usually not listed as specific causes for RPL, every effort should be made to address these issues in patients with RPL. Of specific concern are chronic exposures to toxins including smoking, alcohol, and drugs (Vissenberg and Goddijn, 2011).

2.3.8 Infection:

A number of maternal infections can lead to a single pregnancy loss, including listeriosis, toxoplasmosis, and certain viral infections (rubella, herpes simplex, measles, cytomegalovirus, coxsackie virus). However, there are no confirmed studies to suggest that specific infections will lead to recurrent pregnancy loss in humans. Malaria, syphilis and brucellosis can also cause recurrent miscarriage. (Franssen et al, 2011).

2.4 Antiphospholipid syndrome:
Antiphospholipid syndrome or antiphospholipid antibody syndrome (APS or APLS), or often also Hughes syndrome, is an autoimmune, hypercoagulable state caused by antiphospholipid antibodies. Hughes Antiphospholipid syndrome was described in full in the 1980s, after various previous reports of specific antibodies in people with systemic lupus erythematosus and thrombosis (Hughes, 1983).

The syndrome is sometimes referred to as "Hughes syndrome", after the rheumatologist Graham R.V. (St. Thomas' Hospital, London, UK) who worked at the Louise Coote Lupus Unit at St Thomas' Hospital in London and at the London Lupus Centre at London Bridge Hospital, playing a central role in the description of the condition (Sanna et al, 2006).

APS provokes blood clots (thrombosis) in both arteries and veins as well as pregnancy-related complications such as miscarriage, stillbirth, preterm delivery, and severe preeclampsia.

The diagnostic criteria require one clinical event, i.e. thrombosis or pregnancy complication, and two antibody blood tests spaced at least three months apart that confirm the presence of either lupus anticoagulant, or anti-β2-glycoprotein-I (since β2-glycoprotein-I antibodies are a subset of anti-cardiolipin antibodies, an anti-cardiolipin assay can be performed as a less specific proxy, Antiphospholipid syndrome can be primary or secondary. Primary antiphospholipid syndrome occurs in the absence of any other related disease. Secondary antiphospholipid syndrome occurs with other autoimmune diseases, such as systemic lupus erythematosus (SLE). In rare cases, APS leads to rapid organ failure due to generalized thrombosis; this is termed "catastrophic antiphospholipid syndrome" (CAPS) and is associated with a high risk of death.

Antiphospholipid syndrome often requires treatment with anticoagulant medication such as heparin to reduce the risk of further episodes of thrombosis and improve the prognosis of
pregnancy. Warfarin/Coumadin is not used during pregnancy because it can cross the placenta, unlike heparin, and is teratogenic (Tong et al, 2014).

2.4.1 Signs and symptom of Antiphospholipid syndrome:

The presence of antiphospholipid antibodies (APL) in the absence of blood clots or pregnancy-related complications does not indicate APS.

Antiphospholipid syndrome can cause arterial or venous blood clots, in any organ system, or pregnancy-related complications. In APS patients, the most common venous event is deep vein thrombosis of the lower extremities, and the most common arterial event is stroke. In pregnant women affected by APS, there is an increased risk of recurrent miscarriage, intrauterine growth restriction, and preterm birth (Tong et al, 2014).

A frequent cause of such complications is placental infarctions. In some cases, APS seems to be the leading cause of mental and/or development retardation in the newborn, due to an aPL-induced inhibition of trophoblast differentiation. The antiphospholipid syndrome responsible for most of the miscarriages in later trimesters seen in concomitant systemic lupus erythematosus and pregnancy (Rinne et al, 1998).

Other common findings, although not part of the APS classification criteria, are low platelet count, heart valve disease, and livedoreticularis. There are also associations between antiphospholipid antibodies and headaches, migraines, and oscillopsia (Rinne et al, 2011).

Some studies have shown the presence of antiphospholipid antibodies in the blood and spinal fluid of patients with psychological symptoms (Sokol et al, 2007).

Very few patients with primary APS go on to develop Systemic lupus Erythromatsus (SLE).

2.4.2 Risk factors:
Risk factors for developing antiphospholipid syndrome include:

Primary APS:
- genetic marker HLA-DR7

Secondary APS:
- SLE or other autoimmune disorders
- Genetic markers: HLA-B8, HLA-DR2, HLA-DR3
- Race: Blacks, Hispanics, Asians, and Native Americans

2.4.3 Pathogenesis:

Antiphospholipid syndrome is an autoimmune disease, in which "antiphospholipid antibodies" (anticardiolipin antibodies and lupus anticoagulant) react against proteins that bind to anionic phospholipids on plasma membranes. Like many autoimmune diseases, it is more common in women than in men. The exact cause is not known, but activation of the system of coagulation is evident. Clinically important antiphospholipid antibodies (those that arise as a result of the autoimmune process) are associated with thrombosis and vascular disease. The syndrome can be divided into primary (no underlying disease state) and secondary (in association with an underlying disease state) forms.

Anti-ApoH and a subset of anti-cardiolipin antibodies bind to ApoH, which in turn inhibits Protein C, a glycoprotein with regulatory function upon the common pathway of coagulation (by degradating activated factor V).

Lupus anticoagulant (LAC) antibodies bind to prothrombin, thus increasing its cleavage to thrombin, its active form.
In APS there are also antibodies binding to Protein S, which is a co-factor of protein C. Thus, anti-protein S antibodies decrease protein C efficiency (Triplett, 2002).

Annexin A5 forms a shield around negatively-charged phospholipid molecules, thus reducing their availability for coagulation. Thus, anti-annexin A5 antibodies increase phospholipid-dependent coagulation steps (Rand, 1998).

The Lupus anticoagulant antibodies are those that show the closest association with thrombosis, those that target β2glycoprotein 1 have a greater association with thrombosis than those that target prothrombin. Anticardiolipin antibodies are associated with thrombosis at moderate to high titres (>40 GPLU or MPLU). Patients with both Lupus anticoagulant antibodies and moderate/high titre anticardiolipin antibodies show a greater risk of thrombosis than with one alone.

The increased risks of recurrent miscarriage, intrauterine growth restriction and preterm birth by antiphospholipid antibodies, as supported by invitro studies, include decreased trophoblast viability, syncytialization and invasion, deranged production of hormones and signalling molecules by trophoblasts, as well as activation of coagulation and complement pathways (Tong et al, 2014).

2.4.4 Diagnosis of Antiphospholipid syndrome:

Antiphospholipid syndrome is tested for in the laboratory using both liquid phase coagulation assays (lupus anticoagulant) and solid phase ELISA assays (anti-cardiolipin antibodies).

Genetic thrombophilia is part of the differential diagnosis of APS and can coexist in some APS patients. Presence of genetic thrombophilia may determine the need for anticoagulation therapy. Thus genetic thrombophilia screening can consist of:
-Further studies for Factor V Leiden variant and the prothrombin G20210A mutation, Factor VIII levels, MTHFR mutation.

-Levels of protein C, free and total protein S, Factor VIII, antithrombin, plasminogen, tissue plasminogen activator (TPA) and plasminogen activator inhibitor-1 (PAI-1)

The testing of antibodies to the possible individual targets of aPL such as β2 glycoprotein 1 and antiphosphatidyl serine is currently under debate as testing for anticardiolipin appears to be currently sensitive and specific for diagnosis of APS even though cardiolipin is not considered an in vivo target for antiphospholipid (Miyakis et al, 2006).

2.5 Systemic lupus Erythromatsus:

Systemic lupus erythromatsus (SLE) is multi system autoimmune connective tissue disorder the primarily affect women of child bearing age. the disorder may affect almost all organ in the body it may be mild in some cases for example (involving only skin ) and very sever in other cases(affecting multiple organ including brain, the disease course is characterized by flare (interval of active disease) and Remissions( interval of inactive disease ) (Ringold, 2011).

it is recognize that pregnancy exacerbate SLE and the SLE may increase pregnancy complications, including spontaneous abortion, premature delivery, intrauterine growth restriction IUGR, and preeclampsia, however the other studies found no difference in flare in pregnant and pregnant patients with SLE, and the rate of SLE flare in pregnant have been reported to range from 13-68%, but the rate have been reported to be reduced if pregnancy delayed until disease is quiescent (Ahn et al, 2011).
2.6 Types of Antiphospholipid Antibodies:

2.6.1 False-Positive Test for Syphilis:
In the 1940s, when it was common for people to have premarital exams, doctors realized that some women with lupus tested positive for syphilis. Further studies indicated that 1 in 5 people with lupus had a false-positive syphilis test. The syphilis test of those days—the Wasserman test—was dependant on an antibody found in syphilis patients called reagin. The substance to which this antibody reacts is cardiolipin, so the individuals with a false-positive syphilis test actually had a form of anticardiolipin antibodies. The false-positive syphilis test was the first recognized test for antiphospholipid antibodies, but it is now known that people can have antiphospholipid antibodies without having a false-positive syphilis test and vice versa. The false-positive test is not associated with an increased risk of blood clots in all medical studies performed in the past, but certain studies, including the Johns Hopkins Lupus Cohort, suggest that there is a connection. The false-positive syphilis test was one of the first three recognized indications of antiphospholipid antibodies. The other two were the lupus anticoagulant and anticardiolipin antibody (Wallace et al, 2007).

2.6.2 Lupus Anticoagulant:
In the late 1940s, it was found that an antibody present in some lupus patients prolonged a clotting test dependent on phospholipids. For this reason, it was thought that this antibody increased the tendency to bleed, and thus it was deemed the lupus anticoagulant. However, this name is now recognized as a misnomer for two reasons. First, the term “anticoagulant” is a false label, since lupus anticoagulant actually increases the ability of the blood to clot. Second, the term “lupus” in the name of the antibody is misleading, since more than half of all people who possess this antibody do not have lupus (Wallace et al, 2007).

Tests called coagulation tests are used to detect the lupus anticoagulant (LA). Remember that even though the lupus anticoagulant causes the blood to clot more easily in vivo (i.e., in a person’s body), they actually cause prolonged clotting times in vitro (i.e., in a test tube). Therefore, if it takes more time than normal for the blood to clot, the lupus anticoagulant is usually suspected. The activated partial thromboplastin time (aPTT) is often used to test for LA. If this test is normal, more sensitive coagulation tests are performed, including the modified
Russell viper venom time (RVVT), platelet neutralization procedure (PNP), and kaolin clotting time (KCT). Normally, two of these tests (the apt and the RVVT) are performed to detect whether lupus anticoagulant is present (Wallace et al, 2007).

2.6.3 Anticardiolipin Antibody:
Even though the false-positive syphilis test and the lupus anticoagulant were identified in the 1940s, the link between these entities was not investigated until the 1980s, when a researcher at the Graham Hughes laboratory in Britain named Nigel Harris began looking at antibodies to the phospholipid antigens. Harris realized that cardiolipin was a major element of the false-positive syphilis test, and he developed a more specific test for the antibody. He also determined that the presence of these anticardiolipin antibodies was associated with recurrent thromboses (blood clots) and pregnancy losses. Others in Hughes’ laboratory began to publish studies showing the link between anticardiolipin antibodies and stroke, deep vein thrombosis (DVT), recurrent pregnancy loss, livedo, seizures, and other conditions. In fact, what we now know as antiphospholipid syndrome was known as the anticardiolipin syndrome even though other antiphospholipids, namely the lupus anticoagulant, were known to produce similar effects.

There are different classes (isotypes) of anticardiolipin antibody, namely IgG, IgM, and IgA. IgG is the anticardiolipin antibody type most associated with complications. An enzyme-linked immunosorbent assay (ELISA) is used to test for anticardiolipin antibodies. One can test for all isotypes at once, or they can be detected separately. High levels of the IgMisotype are associated with autoimmune hemolytic anemia, a condition in which an individual’s immune system attacks their red blood cells (Wallace et al, 2007).

2.6.4 Anti-beta2 glycoprotein 1:
Beta2 glycoprotein 1 is the protein in the body to which anticardiolipin antibodies bind, and it is also possible to measure antibodies to beta2 glycoprotein 1. An individual can be positive for anticardiolipin antibodies and negative for anti-ß2 GPI and vice versa, and detection of anti-ß2 GPI is not yet part of routine testing done for patients with an increased likelihood of blood clots (Wallace et al, 2007).

2.7 previous studies:
Many studies has been conducted in Sudan and the world to determine the frequency of antiphospholipid among women with recurrent miscarriage.
In Iran study was conducted in 2004 by Zolghadri, they find out the prevalence of APL is 17.4% and the prevalence of ACL 11.6% (Zolghadri, 2004).

In India 2006 Ghosh in prospective observational study evaluated the prevalence of antiphospholipid syndrome among women with recurrent miscarriage and 27.7% were positive for antiphospholipid antibodies (Ghosh et al, 2006).

In Iraq Amel conducted study on antiphospholipid in Iraqi women with recurrent abortion revealed that the frequency of ACL is 26.5% and insignificant association between age and presence of APL, they significant association between family history recurrent fetal loss and presence of APL antibodies (Amel, et al 2012).

Adel and Ahmed and in Oman 2005 demonstrate the frequency anticarolioplin is 27% among patient of recurrent abortion (Adel and Ahmed, 2005).

In Sudan many studies have been conducted. A study by Abdelnassir in Gezira state revealed that thirteen (26%) were positive of APL antibodies and 11 (22%) are positive for ACL antibodies in out of 50 women with recurrent unexplained miscarriage (Abdelnassir, et al 2014).

Study in 2011 done by Ahmed in Khartoum state conduct study they finished to the frequency of both APL and ACL were (20 %) in women with recurrent miscarriage (Ahmed, et al 2013).

Esam (2013) study the phospholipid as predisposing factor of recurrent miscarriage in Sudanese women they find out the frequency of both APL and ACL is 20% and there are significance correlation between age and presence of APL and ACL (Jevara and Esam, 2013).

Chapter Three

Material and Method

3.1 Study design:

This is cross sectional conducted in Khartoum state, during the period from March 2015 to July 2015.

3.2 Study population and area:
Women with history of recurrent miscarriage, who’s attended to the hospital, during the period from March 2015 to July 2015 will be included, study was done in Omdurman Maternity Hospital, bashiar teaching hospital, National public health laboratory

3.2.1. Inclusion criteria:

Women with a history of three or more consecutive miscarriage, without history of chronic disease and drug use will be included.

3.2.2. Exclusion criteria:

Women with known causes of miscarriage, and women who less than three miscarriage will be excluded.

3.3 Sample size:

Forty women with history miscarriage of who had inclusion criteria selected as study group

3.4 Data collection:

Data will be filled in questionnaires form, including age, number of miscarriages, presence of chronic disease, regular use of drug and family history of miscarriages.

3.5 Ethical consideration:

Ethical clearance was obtained in this study and before sample collection after the agreement of patients whom were informed about the procedure of blood collection and the aim of study.
3.6 Sample collection:

Two ml of blood was collected from superficial vein from study and control group. Under sterile condition, using sterile disposable syringe, was drained into plain container and serum of samples were separated and kept at -20°C. At the end, collected samples were sent to National public health laboratory and level of anti phospholipids and anticardiolipin antibodies in samples was measured by enzyme linked immunoassay test (ELISA).

3.7 Principles:

3.7.1 Antiphospholipid:

Mixture of highly purified cardiolipin, phosphotidyle serine, phosphotidyleinsitolphosphatidic acid and human beta-2-glycoprotein 1 is bound to microwells, antibodies against the coated antigens. If present in diluted patient sample, bind to the respective antigen, washing of the microwells removes unbound unspecific serum and plasma component. 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 (TMB) in the presence of bound conjugate hydrolyze to form blue colour, the addition of an acid stops the reaction forming yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm; the amount of colour is directly proportional to the concentration of antibodies present in the original sample.
3.7.2 Anticardiolipin:

Highly purified cardiolipin is coated on microwells saturated with beta-2-glycoprotein 1, antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen, washing of the microwells removes unbound un specific serum and plasma component horseradish peroxidase (HRP) conjugated anti human antibodies immunologically detect the bound patient antibodies forming a conjugate antibody antigen complex, washing of the micro wells removes un bound conjugate, an enzyme substrate (TMB) in the presence of bound conjugate hydrolyze to form a blue colour, the addition of an acid stops the reaction forming yellow end-product, the intensity of this yellow colour is measured photometrically at 450nm, the amount of colour is directly proportional to the concentration of antibodies present in original sample.

3.8 Material required:

1. Microplate reader
2. Multi-channel pipette for 100µl
3. Vortex mixer
4. Pipettes for 10µl, 100µl and 1000µl
5. Timer
6. Distilled water
7. Measuring cylinder for 100ml and 1000ml
3.9 Procedures:

3.9.1 antiphospholipid:

1- patient samples was diluted 1:100, 990µl of sample buffer in polystyrene tube and 10µl of sample,

2- 100µl of calibrators, controls and prediluted patient samples was Pipetted into the well.

3- incubated for 30 minutes at room temperature (20-28°C).

4 - contents of the microwells was discarded.

5- washed 3 times with 300µl of wash solution.

6 - 100µl of enzyme conjugate was dispensed into each well,

7- incubated for 15 minutes at room temperature.

8 - contents of the micorwells was discarded.

9- washed 3 times with 300µl of wash solution.

10- 100µl of TMB substrate solution was dispensed into each well.

11- incubated for 15 minutes at room temperature.

12- to each well 100µl of stop solution was added.

13- incubated for 5 minutes at room temperature.
14- Optical density was read at 450nm.

3.9.2 Anticardiolipin:

1- patient samples was diluted 1:100, 990µl of sample buffer in polystyrene tube and 10µl of sample,

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14- Optical density was read at 450nm.

3.10 Data analysis:

Data is analyzed using statistical package of social science version (SPSS 11.5)

Relationship will be done by Chi square test and independent t.test using p value of 0.05 to be significance
Chapter Four

Results

Forty sample was collected from women with repeated miscarriage, The age of the study participants ranged from 20-48 years, with mean 30±3.2, the number of miscarriages among study population from 3-8 with mean 4±0.5 (table 4.1)

The results showed that 11 (28%) from participant give positive result for antiphospholipid antibodies, and 29 (72%) give negative result, the percentage of APL among patient 28% (table 4.2)

The frequency of ACL among patient 8 (20%) and 1 (10%) among control give positive results, the percentage of ACL among cases 20% (table 4.3) the total positive cases of antiphospholipid antibodies were 11 (28%) (8 positive for both APL and ACL antibodies and 3 APL alone) (table 4.4).

The serum is determined for both IgG and IgM antibodies, all positive result in case and control in IgM only (table 4.5)

Six (15%) of cases have family history of miscarriage and 34 (85%) have no family history of miscarriage (table 4.6), there was statistically in significance difference in APL and family history chi-square = .120 with p value = 0.729 (table 4.7), there was statistically insignificance difference in ACL and family history with p value = 0.825 (table 4.8).
There was statistically insignificance difference between number of miscarriage and APL result p value =.845 (table 4.9), there was statistically insignificance difference between number of miscarriage and ACL result p value 0.381 (table 4.10).

The female age group (table 4.11) There was statistically insignificance difference between APL result and female age groups p value =0.985 (table 4.12), there was statistically in significance difference between ACL result and female age groups p value =0.963 (table 4.13).

**Table 4.1 Demographic data of test group**

<table>
<thead>
<tr>
<th>Study group</th>
<th>Mean ±SD</th>
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<tbody>
<tr>
<td>Age/ year</td>
<td>30±3.2</td>
</tr>
<tr>
<td>Number of miscarriage</td>
<td>4±0.5</td>
</tr>
</tbody>
</table>

**Table 4.2 Frequency of antiphospholipid antibodies among study group**

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient n(40)</td>
<td>11</td>
<td>28%</td>
</tr>
<tr>
<td>Positive</td>
<td>29</td>
<td>72%</td>
</tr>
</tbody>
</table>
Table 4.3 Frequency of anticardiolipin antibody among study group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient  n(40)</td>
<td>8</td>
<td>20%</td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>80%</td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4 Frequency of positive results among study group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>antiphospholipid alone</td>
<td>3</td>
<td>7.5%</td>
</tr>
<tr>
<td>Antiphospholipid+anticardiolipin</td>
<td>8</td>
<td>20%</td>
</tr>
</tbody>
</table>
Table 4.5 Frequency of positive results according to type of Antibody

<table>
<thead>
<tr>
<th>Type of antibody</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiphospholipid</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>anticadiolipin</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 4.6 Distribution of family history of miscarriage among study group

<table>
<thead>
<tr>
<th>Family history</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>6</td>
<td>15%</td>
</tr>
<tr>
<td>No</td>
<td>34</td>
<td>85%</td>
</tr>
</tbody>
</table>
Table 4.7 Comparison between presence of antiphospholipid antibodies and family history of miscarriage

<table>
<thead>
<tr>
<th>APL</th>
<th>Family history</th>
<th>Total</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>25</td>
<td>29</td>
</tr>
</tbody>
</table>
### Table 4.8 Comparison between presence of anticardiolipin and family history of miscarriage

<table>
<thead>
<tr>
<th></th>
<th>AC L</th>
<th></th>
<th>Total</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>0.825</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>27</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4.9 Comparison between presence of antiphospholipid antibodies and number of miscarriage

<table>
<thead>
<tr>
<th></th>
<th>APL</th>
<th>Number</th>
<th>Mean</th>
<th>SD</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>11</td>
<td>3.82</td>
<td>1.537</td>
<td>0.845</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.10 Comparison between presence of anticardiolipin number of miscarriage

<table>
<thead>
<tr>
<th>ACL</th>
<th>Number</th>
<th>Mean</th>
<th>SD</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>8</td>
<td>4.13</td>
<td>1.727</td>
<td>0.381</td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>3.66</td>
<td>1.234</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.11 Age group distribution among Study group
<table>
<thead>
<tr>
<th>Study group\ years</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 – 30</td>
<td>15</td>
</tr>
<tr>
<td>31 - 40</td>
<td>21</td>
</tr>
<tr>
<td>41- 50</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 4.12 Comparison between age groups of study population and presence of antiphospholipid antibodies

<table>
<thead>
<tr>
<th>APL</th>
<th>Age</th>
<th>Total</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>20-30</td>
<td>31-40</td>
<td>41-50</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>15</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4.13 Comparison between age groups of study population and presence of anticardiolipin

<table>
<thead>
<tr>
<th>ACL</th>
<th>Age</th>
<th>Total</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Chapter Five

**Discussion, Conclusion, Recommendations**

**5.1 Discussion:**

Antiphospholipid syndrome is characterized by recurrent arterial or venous thrombo-embolism or pregnancy loss with association with antibodies directed against anionic phospholipid or protein bound to anionic phospholipids. The aim of this study is to determine the frequency of APL in addition to ACL antibodies in women with recurrent miscarriage and also to determine the association between presence of these antibodies and specific variable like family history, number of miscarriages and age of study group, to carry out this study 40 Sudanese were enrolled, the frequency of

<table>
<thead>
<tr>
<th></th>
<th>20-30</th>
<th>31-40</th>
<th>41-50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>17</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>0.963</th>
</tr>
</thead>
</table>

•...
antiphospholipid antibodies was found 11 (28%) , comparing this result by different studies in Sudan this result in line to the reported frequencies in Sudan by Abdnnassir ( 2014) (26%) , and higher than result obtained by Ahmed (2011) ,and result obtained Jevara and Esam(2013) both studies revealed the frequencies of APL is(20%) , this may be due to varying in number of cases, the result is agree with study in India done by Ghosh frequency (27.7) , and higher than result obtained by Zolghadri in Iran (2004) (17.4%), this may due to varying study area and study population.

The present study showed that the frequency of ACL antibodies is 20% among women with recurrent miscarriage , comparing this with three studies in Sudan ,the result in line of result obtained by Abdelnassir (2014) (22%) ,also it agree and similar to the result obtained by Ahmed (2011) (20%) ,in addition to another result obtained by Jevara and Esam (2013) (20%), another studies in Iran,India ,Iraq and Oman , in Iranian obtained by Zolghadri 2004 the frequency of ACL is (11.6) lower than our result , in Iraq study obtained Amel A et al the frequency (26.5) , Adel A and Ahmed A 2005 in Oman (27%) , Ghosh et al 2006 in india the frequency (27.7).vary in result it’s persistently positive role of the infectious agent which is known predispose to ACL antibody production like syphilis , HIV , cytomegalovirus , hepatitis -c and malaria(Kaushansky and Lichtman, 2010).

In the present study was found insignificance association between family history of recurrent miscarriage and presence of APL antibodies disagree with result obtained by Amel et al that significance association between family history of recurrent fetal loss and presence of APL Revealed that genetic predisposition to development of APL antibodies.

In the present study was found in significance association between female age and presence of APL ,ACL antibodies , this result is agree with result obtained by Amel et al in Iraq(2012) , disagree with another result obtained
by Jevara and Esam in Sudan (2013) which revealed that significant association between age and presence of APL antibodies.

The present study it was found in significance association between number of miscarriages and presence of APL, ACL antibodies.

5.2 Conclusion:

1- Data conclude that presence of APA and ACL in sera of women is associated with recurrent miscarriage.
2- Anti phospholipids antibodies positive cases indicated statistically insignificant association with number of abortions and family history.

3- Insignificance association between APA, ACL and female age was observed

5.3 Recommendations:

1- Further studies are necessary that large sample size is used to give more reliable result.
2-women with recurrent miscarriage should screen for lupus antibodies.

3-every woman with pervious history of unexplained fetal loss should be screened for auto antibodies (APL, ANA, anti-dsDNA).

4-inorder to get more informative data to the recurrent miscarriage patient includes (factorV Leiden, proteinC,proteinS,anti thrombin generation test).

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Appendix I

ΔαμΩή Μή Σή Ά Ό ΔΑ

ΔΦΕ [Μήν ηδόνη] εύλογεύ, εύλογεύ ζεύζο ΔΟΛζ

Δή η καθεστώτική θεολογία μι προ

ΔΕΚΨΗ ηΑ

.................................: Ψηφή

Δτύ Γιά Σή Ά Ό ΔΑ 2υ διπόκη 2Δτύ Γιά Σή Ά Ό ΔΑ 2υ Δτύ Γιά Σή Ά Ό ΔΑ (Μήν) μπαγαζί Δωδεκάτον χρόνο

ΔΕΚΨΗ Δημοκράτικη Υποθήκη Αποφυγή Διαμέσωσης αντιπάλης 6η Ψηφή

ΚΤΒη Γιά Σή Ά Ό ΔΑ 2υ ΔΕΚΨΗ Από Ανάλυση

Δίε Ιη ή Κόσμος 2υ Από Ιη επί Από Ιη Ανάλυση

.................................: Ψηφή

.................................: Ψηφή
Appendix II
Sudan University of Science and Technology
College of Graduate Studies
Faculty of Medical Laboratory Science

Questionnaire

ID NO: ...........................................................................................................

Age: .............................................................................................................

Number of miscarriages: ..............................................................................

Presence of chronic disease:

1/ Yes ( )
2/ No ( )

If yes what is it: ............................................................................................
...................................................................................................................

Regular use of drugs:

1/ Yes ( )
2/ No ( )

If yes what is it: ............................................................................................
...................................................................................................................

Family history of miscarriage:

1/ Yes ( )
2/ No ( )

Signature ..............