CHAPTER ONE
INTRODUCTION

It is well known that the prevalence of several auto antibodies is higher in patients with recurrent miscarriages than in normal women. However, links between individual auto antibodies are unclear. (Mayumi et al., 1999).

The human body has many defence mechanisms against pathogens, one of which is humoral immunity. This defense mechanism produces antibodies (large glycoproteins) in response to an immune stimulus. Many cells of the immune system are required for this process, including lymphocytes (T-cells and B-cells) and antigen-presenting cells. These cells coordinate an immune response upon the detection of foreign proteins (antigens), producing antibodies that bind to these antigens. In normal physiology, lymphocytes that recognize human proteins (autoantigens) either undergo programmed cell death (apoptosis) or become non-functional. This self-tolerance means that lymphocytes should not incite an immune response against human cellular antigens. Sometimes, however, this process malfunctions and antibodies are produced against human antigens, which may lead to autoimmune disease antieardiolipin antibodies often directed against cardiolipin and found in several diseases including syphilis, antiphospholipid syndrome and livedoid vasculitis (Hull et al., 1984).
Autoimmune diseases are conditions in which there is a disorder of the immune system characterized by the abnormal production of antibodies (auto-antibodies) directed against the tissues of the body. Autoimmune diseases typically feature inflammation of various tissues of the body.

Miscarriage usually is loss of pregnancy before viability, define miscarriage as the fetal death in early pregnancy, still birth is fetal death on late pregnancy, miscarriage can divided in two board categories sporadic and recurrent miscarriage. Sporadic miscarriage is the most common complication of pregnancy occurring in up to 15% of all recognized pregnancies (Duckitt and Qureshi, 2011).

Recurrent miscarriage is usually defined as three or more consecutive spontaneous miscarriage; they may or may not follow successful birth. (Duckitt and Qureshi, 2011). Recurrent miscarriage is the major problem affecting 1-2% of women on reproductive age.

While chromosomal aberration, endocrinologicaldysfunction, uterine abnormalities are etiological factors, until recently in most cases, a cause for repregnant loss (RPL) could not be identified. RPL is a well established finding in women with antiphospholipidsyndrome (APS) (Fawad, 2010).

Antiphopholipid antibodies (APL) are heterogeneous family of approximately twenty auto antibodies directed against phospholipids binding plasma proteins. They are associated with systemic thrombsis
including cerebral ischemia, deep vein thrombosis, pulmonary embolism and myocardial infarction. The three most clinically significant are lupus anticoagulant (LA), anticardiolipin antibodies (ACL) and anti-B2 glycoprotein1 antibodies (beta-2-GP1) (Jivraj, 2009).

Antiphospholipid syndrome (APS) is one of the known causes of first and second- trimester recurrent miscarriage. APS is defined as the presence of anticardiolipin antibodies or lupus anticoagulant antibodies, in association with either three or more consecutive fetal losses before week 10 of gestation , one or more premature births before 34 weeks due to severe pre-eclampsia or impaired fetal growth (Duckitt and Qureshi,2011).

1.2 Rationale
Recurrent miscarriage affects 2-5% of population (Wilcoxe et-al.,1988). In Sudan little is known about the prevalence of auto antibodies and related diseases. Accordingly recent study pointed to potential role of anticardiolipin antibodies as possible cause of recurrent miscarriage. The risk of recurrent miscarriage is significantly higher in pregnant women with anticardiolipin antibody and lupus anticoagulant. Women with ACA have3-9 times greater risk of fetal loss than those who had not them. This study aimed to assess serum anticardiolipin antibodies in Sudanese pregnant women and its association with recurrent miscarriage.
2.3 OBJECTIVES

General objective:
To determine levels of anticardiolipin among Sudanese women with recurrent miscarriage.

Specific objectives:
1- To determine recurrent miscarriage among the different groups.
2- To measure serum anticardiolipinIgGantibodies in the study group.
3- To determine the relationship of familyhistory and recurrent miscarriage.
CHAPTER TWO
LITERATURE REVIEW

2.1 Pregnancy

Pregnancy, also known as gravidity or gestation, is the time during which one or more offspring's develop inside a woman. Pregnancy can occur by sexual intercourse or assisted reproductive technology. It usually last around 40 weeks (lunar month) from last menstrual period (LMP) and end in child birth (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 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 the loss of pregnancy before viability. The World Health Organization definition of miscarriage is fetal death in early pregnancy. Stillbirth is fetal death in late pregnancy. At what gestation age miscarriage becomes stillbirth for reporting purposes depends on the country's policy (WHO, 2005).

Miscarriage can be divided into two broad categories – sporadic or recurrent miscarriage. Sporadic miscarriage is the most common complication of pregnancy. It has been estimated that between 12%-15% of all clinically recognized pregnancies miscarry and at least 25% of women will experience at least one sporadic miscarriage in their reproductive lives. The loss of aclinically recognized pregnancy however, only represents the tip of the iceberg of reproductive loose as it is estimated that only 50% of fertilized ova result in the birth of alive child (Jivraj, 2009), whilst sporadic miscarriage is relatively common, recurrent miscarriage the loss of three or more consecutive pregnancies is less common, affecting about 1-2% of the population. Three strands of evidence support the contention that recurrent miscarriage is a distinct clinical entity. (i) firstly the observed incidence of 12% is higher than expected by chance alone of approximately 0.34% this suggest that a persistent underlying cause for their pregnant losses (ii) secondly, a woman risk of having a miscarriage increasing with the number of previous miscarriage they had in one
population, study the risk of miscarriage was highest in women who only had previous miscarriages and lowest in women who only had previous successful pregnancies (22% vs 5%) (Jivraj S, 2009) (iii) thirdly, euploid miscarriages are more common in couples with recurrent miscarriage, a finding that supports an association between non-cytogenetic factors and RM karyotyped specimens obtained from miscarried products of conception, showed an euploid miscarriage rate of 54% among couples with RM vs 37% in a control population from the same institution (Stephenson et al., 2012). Women’s age and reproductive history are two independent factors that affect pregnancy outcome. In study by Rai et al., (1995), conducted in a specialist RM clinic, the future pregnancy outcome of 201 women with a history of unexplained recurrent first trimester miscarriages was determined. No pharmacological treatment was prescribed. Women less than 30 years had miscarriage rate of 25%, 31-35 years had a miscarriage rate of 28% 36-39 years had rate of 33% and <40 years had a miscarriage rate rose to 52% (Jivraj, 2009).

2.3 Causes of miscarriage

Traditional thinking dictates that there is a single cause for miscarriage or recurrent miscarriages. More recently a multi-factorial approach to the problem has been encouraged in such approach, all possible causes of pregnancy loss are considered, and their cumulative effects, when exceeding the threshold, contribute to a miscarriage. (Christian-
Consideration of the timing of the miscarriage is important, as different causes tend to manifest at different periods of gestation. In first trimester miscarriages important causes include chromosomal abnormalities, which occur in about 70% of the cases (Hogge et al., 2003), maternal diseases, including poorly-controlled diabetes mellitus, uncontrolled thyroid diseases, severe systemic lupus erythematosus and antiphospholipid syndrome, poor maternal lifestyle habits (including alcohol consumption, smoking and use of illicit drugs and exposure to non-steroidal anti-inflammatory drugs around the time of conception). Second trimester miscarriages on the other hand, are more commonly caused by specific type of congenital uterine anomalies, cervical incompetence, maternal infection, maternal thrombophilic states, such as inherited thrombophilia and antiphospholipid syndrome, and also chromosomal abnormalities, which account for up to 20% fetal losses during this period (Tien and Tan, 2007).

2.3.1 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 sickle 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 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/PG (invitro fertilization) improves birth rate and structural genetic abnormality, (Franssen, et al 2011).

2.3.2 Anatomical etiology

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, et al, 2005)

The defect in uterus include unicommate, didelphic, bicommate and septate or acute yteri, these anomalies are often detected by MRI or 3-D ultrasounds mentioned above. Septet uterus correct by hysteroscoic-
surgical and no surgical corrective option for the didelphic or unicormuate uterus, (Stephenson and Kutteh, 2007).

The clinical management of pregnancy loss inpatient with uterine fibroids and uterine polyps is also controversial, and there is no evidence that surgical treatment reduces the risk of pregnancy loss, (Stephenson and Kutteh, 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 fibroid and polyps. (RCOG, 2012)

Although more readily associated with second trimester loss 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).

2.3.3 Endocrine Etiologies

Luteal phase defect (LPD), polycystic ovarian syndrome (PCOS), diabetes mellitus thyroid disease, and hyperprolactinemia among the endocrinological disorders implicated in approximately 17% to 20% of
Traditionally, LPD has been proposed to result from inadequate production of progesterone by corpus (Rochat, et al 2008). Luteal and endometrial maturation insufficient for proper placentation. It diagnosed when there is persistent lag of longer than 2 days in 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, et al 2011). Insulin resistance and the resultant hyperinsulinemia is often present in cases of PCOS (as well as type 1 diabetes mellitus) may also play role in RM, as evidenced by the decrease rate of spontaneous pregnancy loss when patients undergo therapy with insulin sensitizing drug, metformin. Poorly controlled type 1 diabetes mellitus is also associated with an increase risk of spontaneous pregnancy loss (Rai and Clifford, 1995).

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, 2012)

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, 2012).

2.3.4 Infection

A number of maternal infections can lead to single pregnancy loss, including liseriosis, toxoplasmosis and certain viral infection (rubella, herpessimplex, measles, cytomegalovirus, coxsackie virus). However, the role of these infections in RM is not clear, with a proposed incidence of 0.5% to 5%. Proposed mechanisms for infection include:(1) direct infection of the uterus, fetus or placenta, (2) placental insufficiency, (3) chronic endometritis or endocervicitis, (4) amnionitis and (5) infected intrauterine device. Infection speculated to play a role in RM include mycoplasma, Urea plasma, Chlamydia trachomatis, L, monocytogenes. (Franssen, et al., 2011).
2.3.5 Thrombophilia
It is possible increased risk factor of RM, the most common problem is the factor V Leiden mutation and prothrombinG20210mutation. 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).

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 and Stephenson, 2006). 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 RM (Williams, 2008).

2.3.6 Lifestyle factors
While lifestyle factors have been associated with increased risk for miscarriage in general, not listed as specific causes for RM. Specific concern are chronic exposures to toxins including smoking, alcohol, ionizing radiation and drugs suggested have cause sporadic RM.

2.3.7 Male factors
Fragmentation may be seen in setting of advanced paternal age or may result from correctable environmental standard semen parameters, including sperm morphology, do not appear to be predictive of
RM (Rubioetal., 2011) sperm aneuploidy and DNA fragmentation have a relation with pregnancy loss, abnormal DNA factors such as exogenous heat toxic exposures, or increase reactive oxygen species in semen (RCOG, 2012).

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

2.3.9 Antiphospholipid antibodies
Antiphospholipid antibodies (APS) comprise a heterogenous group of auto antibodies against negatively charged phospholipids and include lupus anticoagulant (LA) and anticardiolipin antibodies (ACA) (Petrietal., 1998). The importance of these antibodies stems from their established association with thrombosis, thrombocytopenia and recurrent fetal loss. The first well documented association between (APLA) and recurrent fetal loss was reported in 1975 by Nilson and coworkers (Rai; etal, 2010).

2.4 Anti phospholipids syndrome
The antiphospholipids syndrome, also known as Hughessyndrome is autoimmune phenomenon, characterized by multiple different antibodies that are associated with both arterial and venous thrombosis (clots in the arterial and veins) (Empsonetal., 2005).
Historically, aplantibodies were first noted in patients who had positive test for syphilis without signs of infection. Subsequently a clotting
disorder associated 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, In 1983 Dr. Graham Huges described the association between antiphospholipid antibodies and arterial as well as venous thrombosis, (Silveet et al., 2010). In 1990, anticardiolipin autoantibodies were found to require the presence of plasma phospholipids-binding protein B2-glycoprotein1 to bind to cardiolipin. In contrast, anticardiolipin antibodies from patients with syphilis or other infections are 2-glycoprotein 1-independent, binding directly to cardiolipin without requiring a cofactor. As a result of these findings, antiphospholipid autoantibody research has recently focused on phospholipid–binding proteins, rather than phospholipids themselves, with regard to pathophysiology and antibody specificity. The association of antiphospholipid antibodies with thrombosis and pregnancy loss is now well established. With regard to pregnancy loss, antiphospholipid syndrome is an important diagnosis because treatment may improve subsequent pregnancy outcomes and because of potential maternal risks, including thrombosis in pregnancy (Branch, and Khamashtas, 2003).

Antiphospholipid syndrome (APS) was first described in 1986 by HighesHaris and Gharavi as disorder in which antibodies are produced against a variety of phospholipids and phospholipids binding
Clinical manifestations may range from no symptoms to immediately life threatening catastrophic APS. According to international consensus statement on preliminary criteria for classification of antiphospholipids syndrome, a patient which definite APS must have persistent high titers of antiphospholipid antibodies associated with a history of arterial or venous thrombosis or both, or recurrent pregnancy morbidity (Miyakis et al., 2006). Primary APS is defined as presence of APL antibodies in patient with idiopathic thrombosis but evidence of autoimmune diseases. Secondary APS is used when patients with a wide spectrum of autoimmune disorders (primarily SLE and rheumatoid arthritis and thrombosis are also found to have antiphospholipids antibodies) (Baker and Bick, 2008). Clinical manifestations of thrombosis are similar whether APS is primary or secondary. Probable APS is one in which there are typical clinical manifestations but without positive serological test of APL. These are also called-seronegative APS or pre APS. APS is more common in females than males, 5:1 ratio. The antibodies detected for APS used in clinical practice are anticardiolipin (ACL), lupus anticoagulant (LA) (Fawad, 2010). The biological effects mediated by human APL antibodies include reactivity with endothelia structures, with disturbs the balance of prostaglandins E2 and thromboxane production, interaction with platelets with consequent up regulation of platelets aggregation, disregulation of complement activation and interaction of
APL, with phosphatidylserine exposed during trophoblast syncytium formation which cause the possibility of more direct effect of these auto antibodies on placental structure (Mo et al., 2009). The primary antiphospholipid syndrome (PAPS) is defined as the presence of persistently positive titers of APL together with at least one of the following obstetric criteria, (i) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultra sonography or by direct examination of the fetus (ii) One or more premature birth of a morphologically normal neonate before 34th week of gestation because of severe pre eclampsia or eclampsia, or severe placental insufficiency (iii) Three or more unexplained consecutive spontaneous miscarriage before the 10th week of gestation, with maternal anatomical (Jivraj, 2002).

2.5 Beta 2-glycoprotein 1

The major antigen recognized by antiphospholipid autoantibodies is B2-glycoprotein 1 (B2GP1), also also known as apolipo protein H, a member of the complement control protein, or short consensus repeat (SCR), super family. The protein has fishhook shape and binds to anionic phospholipid bilayers through cationic and hydrophobic amino acids in the fifth of its 5 SCR domains, near the carboxyterminus. Recent evidence has indicated that a subset of APL antibodies associated with increased risk of thrombosis and embolism recognize
an epitope in domain 1 of B2GP1 that consists of Gly40-Arg43. It has been suggested that antibody mediated dimerization and pentamerization of B3GP1 increase the affinity/avidity of antibody B2GP1 immune complexes for phospholipids and this increase may be responsible for the pathogenic effects of aPL antibodies. The mechanism of thrombosis in APS and the role of B2GP1 in the process are not yet established. For a recent review of current ideas on the pathogenesis of APS. (Jacob, 2007). Among the proposed mechanisms are several possibilities.

(I) Anti-B2GP1 complexes may interfere with endogenous anticoagulant mechanisms such as crystallization of Annexin A5 anticoagulant shield, fibrinolysis triggered via annexrin A2 and mediated via plasmin, the poteinsC and S mechanism, tissue factor pathway inhibitor, and others

(II) Anti-B2GP1 complexes may trigger signaling events on cells such as blood leukocytes, endothelium, platelets, and trophoblasts that may lead to the expression of prothrombotic and proadhesive phenotypes.

(III) Anti-B2GP1 antibodies may activate complement and trigger an inflammatory reaction on the vascular and/or trophoblast surface (Jacob, 2007).
2.6 Lupus anticoagulant

The lupus anticoagulant (LA), most commonly an immunoglobulin, is an immediate-acting coagulation inhibitor found in a variety of autoimmune disorders and sometimes found in otherwise healthy individuals. It appears to be directed specifically against the phospholipids moiety of prothrombinase complex formed by the interaction of factors Xa, Va, platlet phospholipids active coagulation factors, which slows down the rate of thrombin generation and therefore retards clot formation in vitro, but promotes both venous and arterial thrombosis in vivo. This paradoxical association between in vitro anticoagulant effect and in vivo prothrombotic state activity of this autoantibody is not fully understood. While antiphospholipid syndrome (APS) is known to be one of the most important causes of acquired hyper coagulable states and specifically causes late pregnancy loss, some studies found association of 7% to 10% between recurrent abortions in the first trimester and LA (Olaniyi et al., 2010).

2.7 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a multi-system autoimmune connective tissue disorder that primarily affects women of childbearing age. It is recognized that the pregnancy may exacerbate SLE, and the SLE may increase the pregnancy complications, including spontaneous abortion, premature delivery, intrauterine growth restriction (IUGR), and preeclampsia, however, the other studies found no dif-
ference in flares between pregnant and non-pregnant patients with SLE. The rate of SLE flares in pregnancies have been reported to range from 13-68 %, but rates have been reported to be reduced if pregnancy is de-layed until disease is quiescent (Ahn et al., 2011).

Systemic lupus erythematosus (SLE), also called lupus, is autoimmune disorder in which the body’s immune system (the cell in the body that fight infection) incorrectly attacks the body’s own tissues and organs leading to inflammation and damage. Lupus most commonly affects women of childbearing age but also occur in children, adolescents, and men. The cause of lupus is unknown, but it has been associated with genetic, environmental, and infectious causes. The disorder may affect almost all organs in the body. It may be mild in some cases (for example, involving only the skin) and very severe in other cases (affecting multiple organs, including the brain). The disease course is characterized by flares (intervals of active disease) and remission (intervals of inactive disease). (Ringold et al., 2011).

2.8 Cardiolipin

Cardiolipin is phospholipids (diphosphotidylglycerol) found in inter mitochondrial membrane primarily, but it is also a minor constituent of mammalian membranes in general in diseases with mitochondrial damage. Cardiolipin can start in an antibodies are classes of auto antibodies which have been found in 1-5% of systematically healthy population (Faghihi et al, 2009). This antibodies are also usually detected in
patient with systemic lupus erythematosus (SLE) and anti-phospholipids antibody syndrome (APLS). The increased level of this antibodies has also been observed in several situations including some infection diseases, and has been recognized as sing of APLS as well (Faghihi et al., 2009). In this vein, some recent evidence has shown that bacterial and viral infections have role in etiology of APLS via induction of ACLA production (Firestein et al., 2009). It should be mentioned that APLS patients have tendency to thrombosis but the mechanism is still not transparent. On the other hand, asimilar-ity exists between symptoms of APLS and attributed systemic consequences of periodontal infection such as pro thromboticaccidents, adverse pregnancy outcomes and fetal abortions. While Infectious diseases may have arole in production of ACLA, it has also been suggested that patient with periodontitis might have ahiger level of ACLA in comparison with periodontallyhealthy people. Thus, increased ACLA level might explain the association of such systemic disorders as prothrombotic accidents with periodontitis (Faghihi. et al., 2009). Accordingly, Taylor et al (2006) elimination of periodontitis leads to a decrease in thrombotic and inflammatorymanners which are risk factors for cardiovasculardiseases.
CHAPTER THREE
MATERIALS AND METHODS

3.1 study design
This is a cross sectional study conducted to assess anticardiolipin antibodies in pregnant women in Khartoum State.

3.2 study population and area
Women with history of recurrent miscarriage, who attended to hospitals, during the period from November 2015 to February 2016 were included, blood collection was done in Omdurman Maternity Hospital, Saudi Hospital, and Dr. Ashmige Center for Fertility & Infertility.

3.2.1 Inclusion Criteria
Women with history of three or more consecutive miscarriage as test group. Apparently healthy pregnant women without history of miscarriage were taken as control group.

3.2.2 Exclusion criteria
Women with miscarriage those who had less than three miscarriages.

3.3 Ethical consideration
All the participants were full informed about the aim and benefit of this study and verbal consent was taken from them.

3.4 Data collection and sample process
Data was collected by carefully designed questionnaire. Venous blood samples (5mL) were taken from each participant using disposable syringe, the blood samples were allowed to clot at room temperature and
then serum was obtained after centrifugation at 3000 rpm for 15. The clear serum was withdrawn by means of pipette and transferred to plane container and stored at -20°C till use.

3.5 participants age grouping
The eightythree participants were classified into six age group, less than 20 years, 21-25 years, 26-30 years, 31-35 years, 35-40 years, more than 40 years.

3.6 Measurement of serum Anticardiolipin using ELISA
3.6.1 Principles
Cardiolipin is bound to the micro wells saturated with B2-glycoprotein1. Enzyme-labeled anti-human, IgG immunologically bind to the bound patient antibodies forming conjugate antibody antigen complex. An enzyme substrate in the presence of the bound conjugates is hydrolyzed to form a blue color. The addition of an acid stop the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.
3.6.2 Procedure:

- The reagents and samples were brought to room temperature.
- The reagents were prepared (sample buffer diluted 1:5 with distilled water), (wash buffer diluted 1:50 with distilled water).
- Samples were diluted to 1:101 by sample buffer reagent (1000uL buffer reagent : 10uL serum mix well).
- 100uL of calibrator, positive and negative control and diluted serum sample were added into their corresponding wells.
- The plate was incubated for 30 minutes at room temperature.
- The plate was washed three times with 300 mL phosphate-buffered saline.
- 100uL of enzyme conjugate was added to well under the same condition.
- The plate was incubated for 30 minutes at room temperature.
- The plate was washed three times with 300 mL phosphate-buffered saline.
- 100uL of substrate (hydrogen peroxide plus tetramethylbenzidine in the dark room) was added.
- The plate was incubated for 30 minutes at room temperature, protected from intense light.
- Enzymatic reaction was stopped with 100uL of stopsolution (hydrochloric acid).
- Absorbance read at 450 nm using a micro plate reader.
3.7 Data Analysis:
The data were analyzed using SPSS, version 11.5. The relationship will be done by chi square test using p value of < 0.05 to be significant
CHAPTER FOUR

RESULTS

Eighty three Sudanese women were enrolled in this study; they were classified into six age groups. The frequency of the groups was shown in table (4.1).

Women in age group 31-35 had high frequency of participation compared to other age group (Table 4.1).

The highest frequency of recurrent miscarriage occurred in first and second trimester (44.6% for each).(Table 4.2)

There was no relationship between the disease and family history, 63.9% of women had no family history while 36.1% had family history (Table 4.3).

The frequency of positive anticardiolipin antibody among Sudanese women with recurrent miscarriage was 5(6%) while it was 0.00% in control group.(Table 4.4).

There was significant increase in means of anticardiolipin (2± 0.239) for patient with recurrent miscarriage versus (1.94± 0.00) for control, p value (0.000), (Table 4.5).

Those who were in age group 36-40 year had the high prevalence of anticardiolipin antibodies compared to other group (Table 4.6).
Table (4.1): Frequency of women with recurrent miscarriage according to age group in study group.

<table>
<thead>
<tr>
<th>Age / years</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 20</td>
<td>6</td>
<td>7.2%</td>
</tr>
<tr>
<td>21-25</td>
<td>16</td>
<td>19.3%</td>
</tr>
<tr>
<td>26-30</td>
<td>16</td>
<td>19.3%</td>
</tr>
<tr>
<td>31-35</td>
<td>23</td>
<td>27.7%</td>
</tr>
<tr>
<td>36-40</td>
<td>16</td>
<td>19.3%</td>
</tr>
<tr>
<td>More than 41</td>
<td>6</td>
<td>7.2%</td>
</tr>
<tr>
<td>total</td>
<td>83</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.2): Frequency of trimester of miscarriage among women with recurrent miscarriage

<table>
<thead>
<tr>
<th>Time</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>37</td>
<td>44.6%</td>
</tr>
<tr>
<td>Second</td>
<td>37</td>
<td>44.6%</td>
</tr>
<tr>
<td>Preterm</td>
<td>9</td>
<td>10.8%</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.3): Relationship between recurrent miscarriage and Family history of disease

<table>
<thead>
<tr>
<th>Family history of disease</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>30</td>
<td>36.1%</td>
</tr>
<tr>
<td>No</td>
<td>53</td>
<td>63.9%</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>100%</td>
</tr>
</tbody>
</table>

From table (3-4) we note that no relation between the family history and disease of most of the individuals study are (NO) by (53) and with (%63.90).
Table (4.4): Frequency of anticardiolipin antibodies in study group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n = 83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>6%</td>
</tr>
<tr>
<td>Negative</td>
<td>78</td>
<td>94%</td>
</tr>
<tr>
<td>Control (n = 5)</td>
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<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.5): Comparison between mean of serum anticardiolipin in cases and control groups

<table>
<thead>
<tr>
<th>Result</th>
<th>Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AnticardiolipinGPL/ML Case</td>
<td>2±0.239</td>
<td>0.000</td>
</tr>
<tr>
<td>AnticardiolipinGPL/ML Control</td>
<td>1.94 ±.000</td>
<td></td>
</tr>
</tbody>
</table>

- Chi square test used for comparison
- P value considered significant at level <0.05
Table (4.6): Relationship between Anticardiolipin with recurrent miscarriage according to women age.

<table>
<thead>
<tr>
<th>Age/year</th>
<th>Women with recurrent miscarriage</th>
<th>+ve IgG Anticardiolipin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
<td>%</td>
</tr>
<tr>
<td>Less 20</td>
<td>6</td>
<td>7.2%</td>
</tr>
<tr>
<td>21-25</td>
<td>16</td>
<td>19.3%</td>
</tr>
<tr>
<td>26-30</td>
<td>16</td>
<td>19.3%</td>
</tr>
<tr>
<td>31-35</td>
<td>23</td>
<td>27.7%</td>
</tr>
<tr>
<td>36-40</td>
<td>16</td>
<td>19.3%</td>
</tr>
<tr>
<td>More than 41</td>
<td>6</td>
<td>7.2%</td>
</tr>
<tr>
<td>total</td>
<td>83</td>
<td>100%</td>
</tr>
</tbody>
</table>
This study showed a significant increase in the mean of serum anticardiolipin antibodies in patient group compared to control group respectively $(2.0 \pm 0.239)(1.94 \pm 0.00)$ p.value (0.00). This result agrees with that reported by Ahmed et al, (2004) and Al-Naqdy and Al-Shukaily(2005), who found that there was significant increase in mean of serum anticardiolipin in patient group compared to control group.

In this study, 6% of the patients with recurrent miscarriage had positive anticardiolipin antibodies, compared with 0.00% control group. This result agrees with that reported by Ahmed et al, (2004) who found 19.2% of the patient with recurrent miscarriage with positive anticardiolipin antibodies, compared to 1% in the control group, Al-Naqdy and Al-Shukaily(2005), found that 27% of patient with recurrent miscarriage were positive compared 6% in control group. This indicates that control groups with no history of recurrent abortion can also be positive for anticardiolipin antibodies. The positive control group might be exposed to an antigen that stimulated anticardiolipin antibodies. The high level of anticardiolipin antibodies is known to be harmful to both mother and child, diagnosis and treatment can improve pregnancy outcome (Heilman et al., 2003).
In this study, the highest frequency of recurrent miscarriage (27.7%) occurred in women in age group (31-35) years, this result agrees with Jivarj (2009), who found that women of 31-35 years had a miscarriage rate of 28%. Because the causes of miscarriage are many, we could not know what is the exact cause of each patient. However, we noticed that the highest frequency of RM occur in first and second trimester (44.7%) this agrees with study done by Clifford et al., (1997), which conducted in a specialist RM clinic, the future pregnancy outcome of 201 women with a history of unexplained recurrent first trimester miscarriages was determined. On the other hand, the study revealed that there were no relation between the disease and family history. This result disagrees with Strirrat (2009) who found about 60% of early pregnancy losses associated with sporadic chromosomal anomalies.
5.2 Conclusion
This study concluded that serum anticardiolipin was significantly increased in patients with recurrent miscarriage compared to control group.
Insignificance association between anticardiolipin and patients age was observed.
5.3 Recommendation

1. Further studies are necessary in large sample size to give more reliable result.

2. Every women with history of unexplained fetal loss should be screened for autoantibodies(APL,ANA,anti-DsDNA and LA).
References


13. **Franssen M T, Muster AM, Vander V F, Leschot N JA(2011)** Reproductive out after PGD couples with recurrent abortion, **second edition** 76-90


27. **(R C O G) guidline Royal Collage of Obstetrics and Gynaecology(2012)shoulder dystocia guideline No. 12.”


