Sudan University of Science and Technology

College of Graduate Studies

Stimation of Antiphospholipid and Anticardiolipin Antibodies in Sudanese Women with Recurrent Miscarriage in Khartoum State

تقدير قياس الأجسام المضادة للفوسفات الدهنية و الكارديوليبين فى النساء السودانيات ذوات الإجهاض المتكرر في ولاية الخرطوم

A Thesis submitted in partial Fulfillment for the requirements of the degree of M.Sc. in Hematology and Immunohematology.

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الآيـــــــــــــة

اعوذ بالله من الشيطان الرجيم

بســـم الله الرحمــن الرحيــم

قال تعـــــــــالى:

((اقرأ باسم ربك الذي خلق (1) خلق الانسان من علق (2) اقرا وربك الاكرم (3) الذي علم بالقلم (4) علم الانسان ما لم يعلم (5))).

صدق الله العظيم

العلق (1-5)

Dedication

To my beloved and blessed parents

Who did everything for me Allah's mercy on them

To my husband

Who spent more time encouraging me

To my sons

You will find no better best friend than your mother in this world.

To all patients,

Wishing for them soon healing

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Firstly and foremost, I would like to thank ALLAH for giving me the health and strength which without, I could not have been able to complete this work .

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Abstract

miscarriage is the occurrence of pregnancy loss before 20 weeks of gestation. Its divided into sporadic miscarriage which is most common complication of early pregnancy and recurrent miscarriage which is three consecutive pregnancy loss prior to 20 weeks from last menstrual period. Antiphospholipid Antibodies (APAs) are one of the causes responsible for pregnancy loss.

The aim of study is to measure the levels of antiphospholipid and anticardiolipin antibodies (APL) among women with miscarriage.

his was cross sectional study carried out at Khartoum State, during the period from January to march 2021. Fifty women with miscarriage were participated in this study as case group and compared to other 25 healthy participants as control group. Data was collected through written questionnaire, then specimen of 2ml of venous blood was drawn from each participant and placed in plain container , and tested for antiphospholipid (APL))IgM\IgG) antibodies and anticadiolipn antibodies (ACL) (IgM/IgG) using ELISA assay, Results were analyzed by SPSS (Statistical Package of Social Science version 25).

the results showed that the mean level of antiphospholipid IgM and anticardiolipin antibodies (IgM and IgG) showed significant increase in case group (4.54 , 8.84 , 6.11) when compared to control group(3.05, 2.9, 2.77) with (p. value= 0.016, 0.001, 0.005 respectively), while the mean level of IgG antiphospholipid show no significant difference between case and control group (3.90 versus 3.05 with p value= 0.217). Additionally, The study showed that there was no relationship between the disease and family history.

The study showed that Those who were in age group 30-39 years had the significance high level of antiphospholipid and anticardiolipin antibodies
compared to other group.

This study concludes that there was significant increase in the mean level of antiphospholipid IgM and anticardiolipin antibodies (IgM and IgG) in case group when compared to control group.

المستخلص

الإجهاض هو حدوث فقد الحمل قبل 20 أسبوعًا من الحمل. ينقسم إلى إجهاض متقطع وهو أكثر المضاعفات شيوعًا للحمل المبكر والإجهاض المتكرر وهو فقدان الحمل ثلاث مرات متتالية قبل 20 أسبوعًا من آخر دورة شهرية. تعد الأجسام المضادة للفوسفوليبيد (APAs) أحد الأسباب المسؤولة عن فقدان الحمل.

الهدف من الدراسة هو قياس مستويات الأجسام المضادة للفوسفوليبيد و الكارديوليبين (APL) بين النساء المصابات بالإجهاض المتكرر.

المواد والطرق: أجريت هذه الدراسة المقطعية بولاية الخرطوم خلال الفترة من يناير إلى مارس 2021. وتضمنت هذه الدراسة خمسون امرأة مصابة بالإجهاض المتكرر كمجموعة بحث ومقارنة بـ 25 مشاركة صحية أخرى كمجموعة ضابطة. تم جمع البيانات من خلال استبيان مكتوب . تم سحب 2 مل من الدم الوريدي من كل مشارك ووضعها في حاوية عادية ، تم قياس مضادات الفوسفوليبيد IgM / IgG ((APL)) والكاديوليبين (/ IgM) (ACL IgG) باستخدام اختبار الاليزا ، تم تحليل النتائج بواسطة الحزمة الإحصائية للعلوم الاجتماعية الإصدار 25.

أظهرت النتائج أن متوسط مستوى الأجسام المضادة للفوسفوليبيد IgM والأجسام المضادة للكارديوليبين (IgM و IgG) لها زيادة معنوية في مجموعة الحالات (4.54 ، 8.84 ، 6.11) بالمقارنة مع المجموعة الضابطة (3.05، 2.9 . ، 2.77) مع (القيمة p = 0.016 ، 0.001 ، 0.005 على التوالي) ، بينما لم يُظهر المستوى المتوسط لمضاد الفوسفوليبيد IgG فرقًا معنويًا بين الحالة ومجموعة التحكم (3.90 مقابل 3.05 مع قيمة p = 0.217).

كما اوضحت الدراسة عدم وجود فروقات ذات دلالة احصائية بين المرض والتاريخ العائلي

خلصت هذه الدراسة إلى أن هناك زيادة معنوية في متوسط مستوى الأجسام المضادة للفوسفوليبيد IgM والأجسام المضادة للكارديوليبين (IgM و IgG) في مجموعة الحالة عند مقارنتها بمجموعة التحكم. كما اظهرت الدراسة ان هنالك زيادة ذات دلالة احصائية في متوسط مستوى الأجسام المضادة للفوسفوليبيد و للكارديوليبين في الاشخاص الذين تتراوح اعمارهم بين 30-39 سنة.

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List of abbreviation

ACL	Anticardiolipin antibodies
APA	Antiphospholipid antibodies
APL	Antiphospholipid
APS	Antiphospholipid syndrome
ELISA	Enzyme linked immunosorbant assay
HLA	Enzymes linked immunosorbant assay
RM	Human leuckocytes antigen
PLR	Re pregnant loss
WHO	World health organization
DVT	Deep venous thrombosis

CHAPTER I

Introduction

1.1 Introduction

Miscarriage is the major problem affecting 1-2% of women on reproductive age. It was defined as pregnancy losses before the weak 20 of gestation (robertson *et al*., 2003). Miscarriage can be 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 (Duckittand Qureshi, 2011).

Recurrent miscarriage has been directly associated with genetic factors, hereditary thrombophilia, endocrine and metabolic disorder and uterine abnormalities (Dohont, 2003). 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).

Antiphopholipid antibodies (APL) are heterogeneous family of approximately twenty autoantibodies 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 directed against anionic phospholipids or plasma proteins bound to anionic phospholipids, 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). This study aimed investigate the relationship between antiphospholipid antibodies and miscarriage.

1.2 Rationale

Pregnancy complications are still challenge for gynecologist, because knowledge of pathogenic mechanisms and proteolytic measures are still limited. The measurments of predisposing factors for the development of recurrent miscarriage is useful to determine the incidence of abortion in Sudanese women. Recently, recurrent pregnancy wastage had increased discovery of associations of anti-phospholipid antibodies and anti-cardiolipin antibodies with recurrent pregnancy loss.

In Sudan data is limited 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 objective

To measurments the levels of antiphospholipid and anticardioloipin antibodies among Sudanese women with recurrent miscarriage.

1.3.2 Specific objectives

To estimate the levels of antiphospholipid and anticardioloipin antibodies among Sudanese women with recurrent miscarriage

To compare between results of cases and conrtols.

To correlate between antiphospholipid and anticardioloipin and age and stage of pregnancy.

CHAPTER II

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 (Abam and Steven, 2011).

Pregnancy is typically divided into three trimesters. The first trimester is from week one through twelve and includes conception. 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, follows conception. 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. Stillbirth 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, and most investigator perform diagnostic evaluation after three miscarriage (Bates, 2011).

2.3 Causes of recurrent miscarriage

Traditional thinking dictates that there is a single cause for miscarriage or recurrent miscarriages. More recently, a multifactorial 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. (Christiansen *et al*., 2005) 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 cause include chromosomal abnormalities, which occur in about 70% of the causes (Hogge *et al*., 2003), maternal diseases, including poorly-controlled diabetes mellitus, uncontrolled thyroid diseases, sever 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 205 fetal losses during this period (Tien and Tan,2007).

2.3.1Genetic 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 between6 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 lives birth rate and structural genetic abnormality, (Franssen *et al*., 2011).

2.3.2 Anatomical conditions

2.3.2.1 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. MRI or a combined laparoscopy hysteroscopy of the uterus makes the diagnosis. In addition, uterine leiomyomata could result in pregnancy loss (Strirrat, 2009).

2.3.2.2Cervical 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 (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 (Williums, 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. (Williums, 2012).

2.3.5.1Antiphospholipid 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 antiphospholid syndrome generally take aspirin or heparin in subsequent pregnancies, but questions remain due to the lack of high quality trials (Empson *et al*., 2005).

2.3.5.2Thyroid antibodies

Anti-thyroid autoantibodies 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).

2.3.5.3Increased 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 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

2.3.6.1Reduced 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 Goddijin, 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 Goddijin, 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 Goddijin, 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, cytomegalo virus, 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).

2.4.1 Signs and symptom of Antiphospholipid syndrome

2.4.1.1 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 disloged from a vein below the pulmonary veins and logged in pulmonary vein). Clots in the vein less common in eye, the abdomen, or around brain and liver (Elhassan, 2007).

2.4.1.2 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).

2.4.1.3 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*., 2006).

2.4.2 Pathogenesis of Antiphospholipid syndrome

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 because 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).

2.4.3 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. 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 (Peranettef, 2005).

2.5.1 Primary Hemostasis

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 *et al*., 2006).

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 recoginazation of the internal constitute of the platelets, (Hoffbrand *et al*., 2006).

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 apropotion 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 *et al*., 2006).

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).

2.5.2 Secondary hemostasis

2.5.2.1 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 IXa 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).

2.5.2.2_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 *et al*., 2008).

2.5.2.3_ 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 present 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).

(c) Factor XIII also activated by thrombin in the present of calcium) (Perandettef, 2005).

2.5.3 Fibrinolysis

It is generally acknowledgment that the fibrinolytic system plays an important role in removing fibrin from intravascular and extra vascular sites. Moreover, it is becoming increasing apparent that aberrations of the fibrinolytic response can have catastrophic clinical consequences in terms of both hemorrhagic and thrombosis event. 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 e.g fibrinogen degradation product (FDP) assay (Tagwa *et al*., 2013).

The two main reasons for these short coming have been firstly, the difficulty in reconciling the result 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 chromomeric 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 (Tagwa *et al*., 2013).

2.5.3.1Component 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 (Tagwa *et al*., 2013).

2.6 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).

2.7 Antiphospholipid Antibodies

Antiphospholipid antibodies (APA) comprise a heterogeneous group of autoantibodies directed against negatively charged phospholipids. (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).

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). 1n 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).

2.7.1 Types of Antiphospholipid Antibodies

2.7.1.1 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).

2.7.1.2Lupus 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).

2.7.1.3Anticardiolipin 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).

2.7.1.4Anti-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).

2.8 Previous Studies

In Iran study was conducted in 2004 by zolghadri and his collegus, they find out the prevalence of APL is 17.4% and the prevalence of ACL 11.6 %.

A prospective observational study performed In India at 2006 by Ghosh *et al* evaluated the prevalence of antiphophlipid syndrome among women with recurrent miscarriage and 27.7 % were positive for antiphospholipid antibodies.

In Iraq 2012, Amel *et al* conduct study on antiphospholipd 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.

Adel and Ahmed and in Oman 2005 demonstrate the frequency anticariolipin is 27% among patient of recurrent abortion.

In Sudan, many studies have been conducted. A study by Abdelnassir *et al* at 2014 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.

Study in (2013) 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.

Jevara and 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.

Another study conducted by Yahaya *et al*., (2020) reported that all women patients showed " triple a positive results" of the autoantibodies IgG for aCL, aβ2GPI and lupus anticoagulant (AL). Also, aCL IgG autoantibody was more prevalence in patients than healthy group,

CHAPTER III

Material and Methods

Materials

3.1 Study design

This was cross sectional study.

3.2 Study area

The study was conducted in Khartoum state, .

3.3 Study Duration

The study was conducted, during the period from January to March 2021.

3.4 Study population

Fifty women with history of recurrent miscarriage who had inclusion criteria were selected as study group and compared with 25 healthy women as control group.

3.5. Inclusion criteria: Women with a history of recurrent miscarriage and without history of chronic disease and drug use were included.

3.6. Exclusion criteria: Women who have any disease or disorders can causes miscarriage are excluded.

3.7 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.

3.8 Methods

3.9 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 keep at -20C0 . At the end the collected samples were tested for the level of anti-phospholipids and anticardiolipin antibodies by enzyme linked immunoassay test (ELISA).

3.10 Principles of measurements

3.10.1 Ant phospholipid

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 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 unbound conjugate ,an enzyme substrate(TMB) in the presence of bound conjugate hydrolyze to form blue color ,the addition of an acid stops solution the reaction forming 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 antibodies present in the original sample.

3.10.2. Anticardiolipin

Highly purified cardiolipin is coated on microwells saturated with beta-2glycoprotein 1 , antibodies against the coated antigen , if present in diluted patient sample, bind to the respective antigen ,washing of the microwells removes un –bound 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 proptional to The concentration of antibodies present in original sample.

3.10.3 Data analysis

Data is analyzed using statistical package of social science version (SPSS 25). Qualitative variables were expressed in frequency and percentage using chi-squire test, while quantitative variables were expressed as mean using student T test and One-way ANOVA.

CHAPTER IV

Results

Seventy-five Sudanese women were enrolled in this study, 50 as case group and 25 as control group.). The age of the study participants ranged from 19-49 years, with mean 32.7, the number of miscarriages among study population range more than three times .The highest frequency of recurrent miscarriage occurred in first trimester (figure 4.1).

The study showed that there was no relationship between the disease and family history, 80%of women had no family history while 20% had family history (Table 4.2).

Mean ACA IgM and IgG levels were found to be higher in case group (8.84 , 6.11) as compared to control Group (2.9 , 2.77) however, the difference was found to be statistically significant (p=0.001, 0.005 respectively). The Mean APL IgG levels were found to be higher in case group (3.90) as compared to control Group (3.05) however, the difference was not found to be significant statistically (p=0.217). Mean ACA IgM levels were found to be higher in case group (4.54) as compared to control group (3.05) and the difference between two groups was also found to be significant statistically (p=0.016) as shown in (table 4.3).

The study showed that those who were in age group 30-39 years had the significance high level of antiphospholipid and anticardiolipin antibodies compared to other group (Table 4.4).

Table (4.1): Frequency of trimester of miscarriage among women with recurrent miscarriage

	Frequency	Percentage
Frist	23	46%
Second	20	40%
Third	7	14%

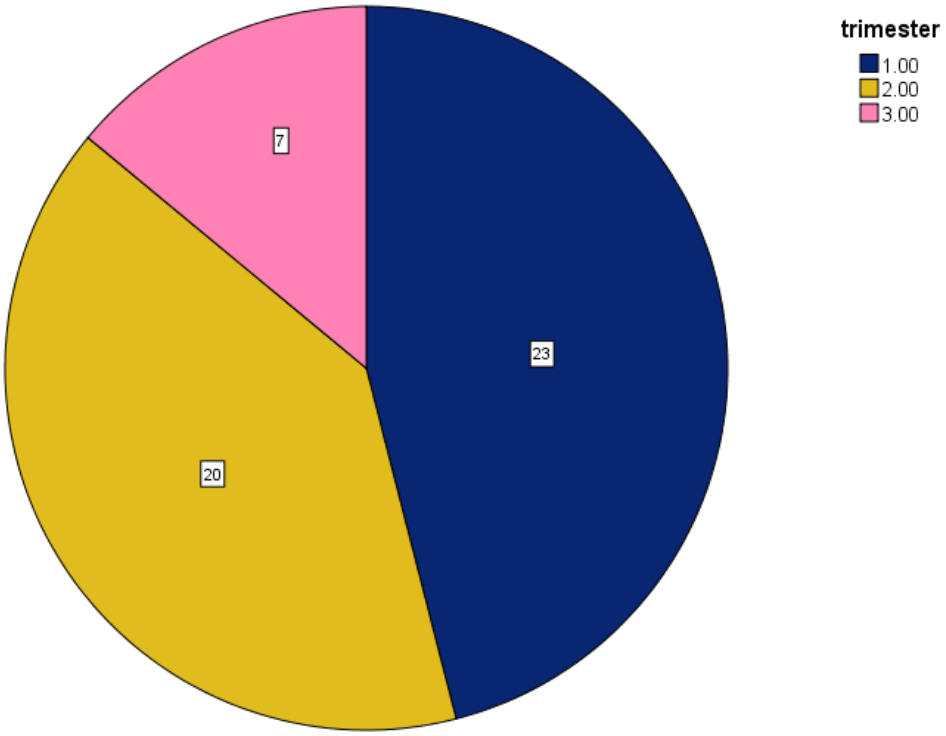

Figure 4.1: trimester of women with recurrent miscarriage

Table (4.2): Relationship between recurrent miscarriage and Family history of disease

	Frequency	Percentage	P value
Yes	10	20%	0.84
No	40	80%	

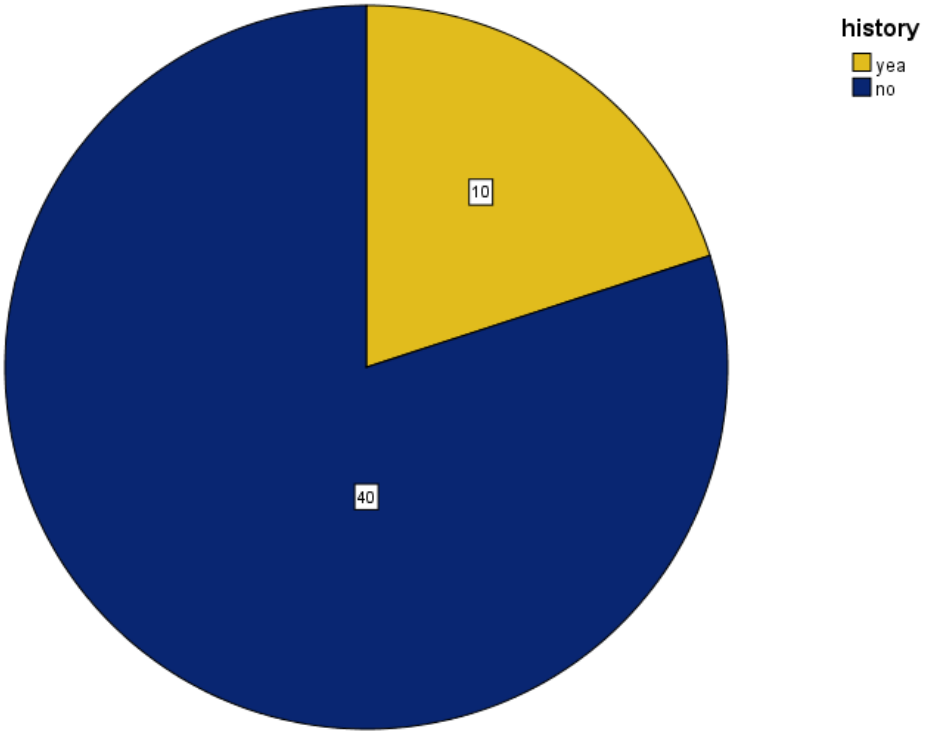

Figure 4.2: Frequency of family history among case group

Table (4.3): Average distribution of Antiphospholipid and anticardiolipin antibodies in-patient and control

Parameters	Case group (n=50)	Control group (n= 50)	p. value
APL IgM	4.54	3.05	.016
APL IgG	3.90	3.05	.217
ACL IgM	8.84	2.9	.001
ACL IgG	6.11	2.77	.005

Table (4.4): Average distribution of Antiphospholipid and anticardiolipin based on age

	APL IgM	APL IgG	ACL IgM	ACL IgG	P value
19 - 29	3.5263	2.4579	10.3	6.10	.242
30 - 39	4.9200	4.98	9.16	7.06	.046
40 - 49	5.61	4.42	5.7	4.4	.396

CHAPTER V

Discussion, conclusion & Recommendations

5.1 Discussion

Recurrent Miscarriage involves the loss of a pregnancy, usually within the first three months of conception in which many factors play a role such as genetic, hormonal disorders, maternal age, uterine factors, and the infections, environmental and immunologic factors (Gracia *et al*., 2005). Antiphospholipid & Anticardiolipin antibodies are associated with placental vascular thrombosis, thrombocytopenia, intervillous fibrin deposition, and placental infarction, leading to the cause of fetal losses (Kupferminc MJ, 2003). Hence, the aim of the present study was to measure the levels of Antiphospholipid & Anticardiolipin (IgG and IgM antibodies) among women with a recurrent miscarriage in Khartoum state, Sudan.

The study showed that there was no relationship between the disease and family history. This finding agree with the study of Amel *et al* who found insignificance association between family history of recurrent miscarriage and presence of APL antibodies Revealed that genetic predisposition to development of APL antibodies.

In this study, the mean level of antiphospholipid IgM and anticardiolipin antibodies (IgM and IgG) showed significant increase in case group when compared to control group (p. value > 0.05), while the mean level of IgG antiphospholipid show no significant difference between two group. These finding is quite similar to study of Nilam *et al*., in india (2018), Zolgadri *et al*., in Iran (2007) and Velayathrabhu *et al*., in India (2009) who reported that women with unsuccessful pregnancies had mounted levels of APA and ACA. The elevated levels of these autoantibodies may be related to the occurs of chronic infection which initiates the autoimmune disease. as a result of infection for long time with treatment, leads to induce the formation of antibodies that contribute to the pathogenesis of disease through the deposition of immune complexes which trigger the autoimmune reactions.

the present study found significance difference in the mean levels APL ,ACL antibodies among female of age group (30-39 years), this result agree with results obtained by Jevara and Esam in Sudan (2013) and by Ibrahim *et al* in iraq

(2020) Which revealed that there was significant association between age and presence of APL and ACL antibodies with higher levels of antibody positivity in patients older than 30-year-old & disagree with another result obtained by Amel *et al* in Iraq (2012), who found association between female age and presence of APL ,ACL antibodies. This discrepancy could be due to genetic, environmental factors, and pathological mechanisms. Further studies are needed to explore this.

5.2 Conclusion

This study concludes that there is a significant increase in the mean level of antiphospholipid (IgM) and anticardiolipin antibodies (IgM and IgG) in case group when compared to control group, while the mean level of antiphospholipid type IgG shows no significant difference between two groups. All study parameters show no significant difference according to family history.

5.3 Recommendations

- This study concludes that Data of this study suggest that APL and ACL Abs should be included in the laboratory diagnosis of miscarriage women or in women having strong family history of miscarriage for better gestational results.

- Further studies are needed to investigate the relationship between autoantibodies positivity and pregnancy outcomes recruiting samples from all over Sudan.

- Administration of anticoagulents is recommended, in women with APS should begin as soon as pregnancy confirmed.

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Appendixes

Appendix (1) Questionnaire

Age: _____

Years:

19- 29 ( ) 30- 39 ( ) 40 - 49 ( )

Trimesters:

First ( ) Second ( ) Third ( )

Number of miscarriage:

……

Family history

Yes ( ) No ( )

Laboratory investigation

Test	APL IgM	APL IgG	ACL IgM	ACL IgG
Results				

Appendix (2)

ELISA washer

Appendix (3)

ELISA Reader