

Chapter one

1. Introduction and Literature Review

1.1. Introduction

The lupus anticoagulant interferes with lipoprotein-dependent stages of coagulation and is usually detected by prolongation of the APTT test. This inhibitor is detected in 10% of patients with systemic lupus erythematosus (SLE) and in patients with other autoimmune diseases who frequently have antibodies to other lipid - containing antigens, e.g. cardiolipin. The antibodies is not associated with a bleeding tendency but there is an increased risk of thrombosis with other causes of thrombophilia, an association with recurrent miscarriage (Hoffbrand *et al*, 2001). Miscarriage (abortion) is the involuntary loss or termination of pregnancy prior to 24 weeks' gestation. Other definitions include the loss of pregnancy before viability. The World Health Organization definition is 'expulsion or extraction of an embryo or fetus weighing 500 g or less from its mother' (Barry. *et al*, 2012). Recurrent pregnancy loss (RPL) is the loss of 3 or more spontaneous consecutive pregnancies (Festin. *et al*, 1997). The aim of this study is investigate lupus anticoagulant among Sudanese women with habitual abortion, Furthermore, to evaluate the titer of circulating anticardiolipin antibodies which have been reported as being strongly associated with lupus anticoagulant. there is no well established screening test for lupus anticoagulant that can be used for screening of high risk group, and hence help in reducing LA morbidity. Identification of associated assay marker can play an important role in identification of patients that should go for confirmatory test and thus may limit the use of expensive test.

The current study showed that the age group most affected is between 28-33 years comprising 27.9% of the study group, and lowest affected age group is between

40-45 years comprised 5.0% of the study group as show in table (1). The majority of patient was came from South Kurdfan state 23(16.4%) and the least were from Blue Nile & Red Sea 0(0.0%) show in table (2). In the presence of LA and ACA in women with habitual abortion were significantly associated with habitual abortion and the age. LA is a significant cause of recurrent fetal loss in Sudanese women. The dRVVT is the best screening test for LA. The LA was not detected in control group. Women with habitual abortion should be screened for LA using dRVVT test as screening coagulation test , ACA IgG test using ELISA method. Women with habitual abortion should be routinely screened for coagulation tests It is there for advisable to routinely use the test (dRVVT) when screening for LA. Women with habitual abortion should be informed about risk factor of coagulation during pregnancy.

1.2. Literature review:

1.2.1. Normal homeostasis:

The platelet membrane has integral glycoproteins essential in the initial events of adhesion and aggregation, leading to formation of the platelet plug during haemostasis. Glycoprotein receptors react with aggregating agents such as collagen on the damaged vascular endothelial surface, fibrinogen, and von Willebrand factor to facilitate platelet and platelet-endothelial cell adhesion. The major glycoproteins are the Ib-IX complex, whose main binding protein is von Willebrand factor, and IIb/IIIa which specifically binds fibrinogen. Storage organelles within the platelet include the “dense” granules which contain nucleotides, calcium and serotonin, and α -granules containing fibrinogen, von Willebrand factor, platelet-derived growth factor and many other clotting factors. Following adhesion, the platelets are stimulated to release the contents of their granules essential for platelet aggregation. The platelets also provide an extensive phospholipid surface for the interaction and activation of clotting factors in the coagulation pathway. (Drew, 2003).

1.2.2. Blood coagulation:

At sites of vascular damage, circulating platelets adhere to sub endothelial structures and undergo a series of reactions, which lead to primary hemostasis due to the formation of a platelet plug. Concomitant to these events, the subendothelial membrane protein tissue factor (TF) is exposed to blood. A small amount of activated factor VII (FVIIa), present in circulating blood, binds to TF and triggers a series of proteolytic reactions which culminate in the formation of thrombin and the conversion of fibrinogen to insoluble fibrin. FVIIa bound to TF specifically cleaves and activates the two vitamin K-dependent plasma proteins, factor IX (FIX) and factor X (FX). Activated FX (FXa) activates prothrombin to thrombin,

whereas activated FIX (FIXa) activates FX. Both FIXa and FXa are poor enzymes that require protein cofactors, calcium ions and negatively charged phospholipid surfaces for the expression of their full biological activity. The protein cofactors for FIXa and FXa are the activated forms of factor VIII (FVIIIa) and factor V (FVa), respectively. As a result of multiple protein—protein and protein—phospholipid interactions, enzymatically highly efficient complexes are assembled on the phospholipid surface. The initiation of blood coagulation by TF is usually referred to as the extrinsic pathway or the TF pathway. In association with injury, this is the physiologically most important mechanism of blood coagulation. However, coagulation can also be activated through the *intrinsic pathway*, which is triggered by the activation of the contact phase proteins (FXII,FXI, prekallikrein and high molecular weight kininogen) that follows upon exposure of blood to certain negatively charged surfaces. The intrinsic pathway does not appear to be physiologically important for injury-related coagulation *in vivo*; this is illustrated by the lack of bleeding problems in individuals with deficiency of FXII. Thrombin generated at sites of vascular injury expresses a number of procoagulant properties. It amplifies the coagulation process by activating FXI and in addition it activates platelets and converts fibrinogen to fibrin. Moreover, in a positive feedback reaction, thrombin converts the procofactors FV and FVIII into their biologically active counterparts (FVa and FVIIIa). (Drew *et al*, 2005).

1.2.3. Classification of Coagulation Factors:

Coagulation factors may be categorized into substrates, cofactors, and enzymes. Substrates are the substance upon which enzymes act. Fibrinogen is the main substrate. Cofactors accelerate the activities of the enzymes that are involved in the cascade. Cofactors include tissue factor, factor V, factor VIII, and Fitzgerald factor. All of the enzymes are serine proteases except factor XIII, which is a

transaminase. There are three groups in which coagulation factors can be classified:

1. The fibrinogen group consists of factors I, V, VIII, and XIII. They are consumed during coagulation. Factors V and VIII are labile and will increase during pregnancy and inflammation.
2. The prothrombin group: Factors II, VII, IX, and X all are dependent on vitamin K during their synthesis. This group is stable and remains preserved in stored plasma.
3. The contact group: Factor XI, factor XII, prekallikrein, and high-molecular-weight kininogen (HMWK) are involved in the intrinsic pathway, moderately stable, and not consumed during coagulation.

Factor I, Fibrinogen:

Substrate for thrombin and precursor of fibrin, it is a large globulin protein. Its function is to be converted into an insoluble protein and then back to soluble components. When exposed to thrombin, two peptides split from the fibrinogen molecule, leaving a fibrin monomer to form a polymerized clot.

Factor II, Prothrombin:

Precursor to thrombin, in the presence of Ca^{2+} , it is converted to thrombin (IIa), which in turn stimulates platelet aggregation and activates cofactors protein C and factor XIII. This is a vitamin K-dependent factor.

Factor III, Thromboplastin:

Tissue factor activates factor VII when blood is exposed to tissue fluids.

Factor IV, Ionized Calcium:

This active form of calcium is needed for the activation of thromboplastin and for conversion of prothrombin to thrombin.

Factor V. Proaccelerin or Labile Factor:

This is consumed during clotting and accelerates the transformation of prothrombin to thrombin. A vitamin K-dependent factor, 20% of factor V is found on platelets.

Factor VI, Nonexistent:

Factor VII, Proconvertin or Stable Factor:

This is activated by tissue thromboplastin, which in turn activates factor X. It is a vitamin K-dependent factor.

Factor VIII, Antihemophilic:

This cofactor is used for the cleavage of factor X—Xa by IXa. Factor VIII is described as VIII/vWF VIII:C active portion, measured by clotting, VIII:Ag is the antigenic portion, vWFAg measures antigen that binds to endothelium for platelet function; it is deficient in hemophilia A.

Factor IX, Plasma Thromboplastin Component:

A component of the thromboplastin generating system, it influences amount as opposed to rate. It is deficient in hemophilia B, also known as Christmas disease. It is sex linked and vitamin K- dependent.

Factor X, Stuart-Powers:

Final common pathway merges to form conversion of prothrombin to thrombin, activity also related to factors VII and IX. It is vitamin K-dependent and can be independently activated by Russell' s viper venom.

Factor XI, Plasma Thromboplastin Antecedent :

Essential to intrinsic thromboplastin generating of the cascade, it has increased frequency in the Jewish population. Bleeding tendencies vary, but there is the risk of postoperative hemorrhage.

Factor XII, Hageman factor:

This surface contact factor is activated by collagen. Patients do not bleed but have a tendency to thrombosis.

Factor XIII, Fibrin Stabilizing Factor:

In the presence of calcium, this transaminase stabilizes polymerized fibrin monomers in the initial clot. This is the only factor that is not found in circulating plasma.

High-Molecular-Weight Kininogen:

This surface contact factor is activated by kallikrein.

Prekallikrein, Fletcher Factor:

This is a surface contact activator, in which 75% is bound to HMWK (Betty.2007).

Table (1.1) Coagulation Factors and Related substances

Number and/ or name	Function	Associated genetic disorders
I (Fibrinogen)	Forms clot (fibrin)	Congenital Afibrogenmia
II (Prothrombin)	Thrombin or IIa activates I,V,VII, VIII, XI, XIII, Protein C and platelets	Familial Renal Amyloidosis
III (Tissue factor)	Co – factor of VIIa	Thrombophilia
IV Calcium	Required for coagulation factors to bind to phospholipid (formerly known as factor IV)	
V (Proaccelerin)	Co- factor of X with which forms the prothronbinase complex	Hypocalcemia is not associated with clotting problem because it has other more serious effects.
VI	Unassigned – old name of factor Va	Activated protein C resistance
VII (stable factor, proconvertin)	Activates IX,X	Congenital proconvertin/ factor VII deficiency
VIII (Anti Hemophilic Factor A)	Co-factor of IX with which it forms the TENASE complex	Haemophilia A
IX (Antihemophilic factor B or Christmas factor)	Activates IX	Haemophilia B
X (Stuart-Power factor	Activates IX	Congenital Factor X deficiency
XI (Plasma Thromboplastin Antecedent)		Haemophilia C
XII (Hageman factor)	Activates factor XI,VII and prekallikrein	Hereditary Angioedema type III
XIII (Fibrin – Stabilizing Factor)	Congenital Factor XIIIa/b deficiency	
Von Willebrand factor of vWF	Binds to VIII, mediates platelet adhesion	Von willebrand disease
Prekallikrein (Fletcher factor)	Activates XII and prekallikrein; cleaves HMWK	Prekallikrein/ Fletcher factor deficiency
High Molecular weight Kininogen or HMWK (Fitzgerald factor)	Supports reciprocal activation of XII,XI. And prekallikrein	Kininogen deficiency
Fibronectin	Mediates cell adhesion	Gomerulopathy with fibronectin deposits.
Antithrombin III	Inhibits IIa, Xa, and other proteases	Antithrombin III deficiency
Protein C	Inactivates Va and VIIIa	Protein C Deficiency
Protein S	Cofactor for activated protein C	Protein S deficiency
Protein Z	Mediates thrombin adhesion to phospholipids and srimulates degradation of factor X by ZPI	Protein Z deficiency
Plasminogen	Converts to plasmin , lyses fibrin and other proteins	Plasminogen deficiency, type I (ligneous conjunctivitis
Alpha2 antiplasmin	Inhibits plasmin	Antiplasmin deficiency
Tissue plasminogen activator or tPA	Activates plasminogen	Thrombophilia
Urokinase	Activates plasminogen	Quebee platelet
Plasminogen Activator Inhibitor 1 (PAI1)	Inactivates tPA&urokinase (endothelial PAI)	Plasminogen activator inhibitor-1 deficiency
Plasminogen Activator Inhibitor 2 or PAI2	Inactivates tPA&urokinase	
Cancer procoagulant	Pathological factor X activation	Thrombophilia

1.2.4. Endothelial cells:

The endothelial cell has an active role in the maintenance of vascular integrity. This cell provides the basement membrane that normally separates collagen, elastin and fibronectin of the subendothelial connective tissue from the circulating blood. Loss or damage to the endothelium results in both haemorrhage and activation of the haemostatic mechanism. The endothelial cell also has a potent inhibitory influence on the haemostatic response, largely through the synthesis of PGI₂ and NO, which have vasodilatory properties and inhibit platelet aggregation. In contrast, endothelins are a family of vasoactive peptides that can activate fibrinolysis via the release of tPA. Synthesis of tissue factor which initiates haemostasis only occurs in endothelial cells following activation and its natural inhibitor, tFPI, is also synthesized. Synthesis of prostacyclin, VWF, plasminogen activator, antithrombin and thrombomodulin, the surface protein responsible for activation of protein C, provides agents that are vital to both platelet reactions and blood coagulation (Hoffbrand. *et al*, 2001)

1.2.5. Fibrinolysis:

It is widely acknowledged that the principal functions of the fibrinolytic system are to ensure that fibrin deposition in excess of that required to prevent blood loss from damaged vessels is either prevented or rapidly removed (i.e. that a localized procoagulant response is achieved without compromising blood circulation generally) and following re-establishment of haemostasis. An existing fibrin mesh is later removed as part of the process of tissue remodelling. The system of pro- and antifibrinolytic factors that has evolved to meet these requirements is closely coupled to that which results in fibrin clot formation. Fibrinolysis is essentially a localized, surface-bound phenomenon, with most events being catalysed by the presence of cross linked fibrin itself, i.e. 'fibrin orchestrates its own destruction'.

For this reason, the assays of fibrinolytic factors carried out in the soluble phase, in particular in systemic blood, may be misleading and should be interpreted with great caution. (Hoffbrand. *et al*, 2003).

1.2.6. Components of the fibrinolytic system:

These include plasminogen (PLG) and plasmin, several endogenous (tissue or plasma-derived) or exogenous (e.g. bacterial or venom derived) PLG activators, and a number of inhibitors of plasmin or of the PLG activators. Both endogenous and exogenous fibrinolytic factors have been used clinically to treat venous and arterial thrombosis, with varying degrees of success (Hoffbrand. *et al*, 2003).

1.2.7. Inhibitors of fibrinolysis:

The plasmin-generating potential of plasma is sufficient to degrade completely all of the fibrinogen in the body in a very short period of time. It is prevented from doing so by the PLG activator inhibitors or PAIs, most of which belong to the serpin family, and by a number of circulating inhibitors of plasmin itself (the antiplasmins) (Hoffbrand. *et al*, 2003).

1.2.8. The Vitamin K-Dependent Factors:

The vitamin K-dependent factors group includes coagulation factors II, VII, IX, and X. However, it is important to remember that the anticoagulant proteins S, C, and Z are also vitamin K-dependent. Each of these proteins contains a number of glutamic acid residues at its amino terminus that are γ -carboxylated by a vitamin K-dependent mechanism. This results in a novel amino acid, γ -carboxyglutamic acid, which is important in promoting a conformational change in the protein that promotes binding of the factor to phospholipid. Because this binding is crucial for final common pathway merges to form conversion of prothrombin to thrombin, activity also related to factors VII and IX. It is vitamin K-dependent and can be independently activated by Russell's viper venom (Dacie. *et al*, 2001) .

1.2.9. Fibrinogen:

Fibrinogen is a large dimeric protein, each half consisting of three polypeptides named A α , B β , and γ held together by 12 disulphide bonds. The two monomers are joined together by a further three disulphide bonds. A variant γ' chain denoted γ' is produced by a variation in messenger RNA splicing. In the process a platelet binding site is lost and high-affinity binding sites for FXIII and thrombin are gained. The γ' variant constitutes approximately 10% of plasma fibrinogen. A less common (<2%) α chain variant “ γ E” is also produced by splice variation. Fibrinogen is also found in platelets, but the bulk of this is derived from glycoprotein IIb/IIIa-mediated endocytosis of plasma fibrinogen, which is then stored in alpha granules, rather than synthesis by megakaryocytes. Fibrin is formed from fibrinogen by thrombin cleavage of the A and B peptides from fibrinogen. This results in fibrin monomers that associate to form a polymer that is the visible clot. The central E domain exposed by thrombin cleavage binds with a complementary region on the outer or D domain of another monomer. The monomers thus assemble into a staggered overlapping two-stranded fibril. More complex interactions subsequently lead to branched and thickened fibre formation (Dacie. *et al* ,2001) Fibrinogen is Produced by liver, it is an acute phase protein, raised in inflammatory reactions, pregnancy, stress, etc. Converted into fibrin by the action of thrombin and is a key component of a clot. Abnormalities of fibrinogen are more often acquired than inherited. Inherited defects are usually quantitative and include heterozygous hypofibrinogenaemia or homozygous afibrinogenaemia. Qualitative defects-the dysfibrinogenaemias-are inherited as incomplete autosomal dominant traits with >200 reported fibrinogen variants; defective fibrin polymerisation or fibrinopeptide release may occur. Most patients are heterozygous. (Drew. *et al* ,2004).

1.2.9.1. Clinical presentation:

Symptoms of bruising, bleeding usually after trauma or operations will depend on the concentration and are more severe when $<0.5\text{g/L}$. Afibrinogenaemia (fibrinogen $<0.2\text{g/L}$) is a severe disorder with spontaneous bleeding, cerebral and gastrointestinal haemorrhage and haemarthrosis. It may present as haemorrhage in the newborn. Recurrent miscarriages occur. Most patients with dysfibrinogenaemia are heterozygous and bleeding symptoms are usually minor; arterial and venous thrombosis is described with some variants (Drew. *et al.*, 2004).

1.2.10. The Role of Fibrinogen in Hemostasis:

Fibrinogen is the principal substrate of the coagulation and fibrinolytic system. This clotting factor has the highest molecular weight of all of the clotting factors, and it is the substrate upon which the coagulation system is centered. This factor is heat labile but stable in storage. When fibrinogen is transformed to fibrin under the influence of thrombin, it is the onset of solid clot formation. The formation of fibrin occurs within minutes due in part to a positive feedback mechanism within the hemostasis system. Once clotting factors are activated, they accelerate the activity of the next factor, pushing the reaction to conclusion. Negative feedback occurs when the activity of the reaction is delayed. This is the role played by naturally occurring inhibitors within the hemostatic system. With the assistance of factor XIII and thrombin, the fibrinogen molecule is stabilized by cross—linked fibrin. Within hours, the fibrinolytic system swoops in to dissolve the clots that have formed and to restore blood flow. The creation of cross—linked fibrin is an orderly process by which fibrinogen is cleaved into fibrinopeptides A and B by thrombin. Fibrinogen is composed of three pairs of polypeptide chains: alpha, beta, and gamma. When thrombin is generated, it cleaves small portions of the alpha and beta chains, creating fibrinopeptides A and B. The remaining portions of the alpha

and beta chains stay attached to the fibrinogen molecule. With fibrinopeptides A and B cleaved, the fibrin monomer is created. These monomers spontaneously polymerize by hydrogen bonding to form a loose fibrin network, which is soluble. Trapped within the soluble clot are thrombin, antipplasmins, plasminogen, and tissue plasminogen activator (tPA). Because thrombin is now protected from its inhibitors, it activates factor XIII and calcium and then catalyzes the formation of peptide bonds between monomers, forming fibrin polymers that lead to an insoluble and resistant clot. Balance between the coagulation and fibrinolytic systems are critical for maintenance of circulation and injury repair. An imbalance in the coagulation system could cause excess clotting; an imbalance of the fibrinolytic system could cause hemorrhaging. Several other components may play a role in hemostatic balance. In early studies, it has been suggested that individuals with a high concentration of lipoprotein A may have reduced fibrinolytic activity due to decreased plasmin generation. Cholesterol and triglycerides are all fatty components of lipoproteins. It is conceivable that reduced plasmin generating activity in individuals with increased levels of lipoprotein will lead to less clot dissolution, leaving clots available for a pathological outcome (Betty. 2007).

1.2.11. Disorders of Fibrinogen:

Appropriate levels of fibrinogen are necessary to maintain hemostasis and to cause platelets to aggregate. The reference range for fibrinogen is 200 to 400 mg/dL. Fibrinogen is an acute- phase reactant, meaning that there will be a transient increase in fibrinogen during inflammation, pregnancy, stress, and diabetes and when taking oral contraceptives. Therefore, a careful patient history is necessary when evaluating a problem involving fibrinogen. For the most part, decreases in fibrinogen result from acquired disorders such as acute liver disease, acute renal disease, or disseminated intravascular coagulation. Acquired increases in

fibrinogen may be demonstrated in hepatitis patients, pregnant patients, or those with atherosclerosis. The inherited disorders of fibrinogen are afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia. These conditions are rare and are marked by hematomas, hemorrhage, and ecchymoses depending upon severity (Betty. 2007).

1.2.11.1. Afibrinogenemia:

The homozygous disorder, afibrinogenemia, is an autosomal recessive disorder that shows less than 10 mg/dL fibrinogen in the plasma. This small amount of fibrinogen is usually not demonstrable by traditional methods. Infants with afibrinogenemia will show bleeding from the umbilical stump; poor wound healing and spontaneous abortion are also features of this disorder. Laboratory results will show elevated PT, aPTT, thrombin time (TT), reptilasetime, and abnormal platelet aggregation with most aggregating agents and elongated bleeding time. Cryoprecipitate and fresh frozen plasma are the replacement products used for medical management of bleeds for these patients (Betty . 2007).

1.2.11.2. Hypofibrinogenemia:

Hypofibrinogenemia is the heterozygous form of afibrinogenemia. This disorder is autosomal recessive and patients show between 20 and 100 mg/dL fibrinogen in their plasma. Patients with this disorder may show mild spontaneous bleeding and severe postoperative bleeding. Results of laboratory testing, whether prolonged or normal will depend on the amount of fibrinogen present (Betty. *et al* , 2007).

1.2.11.3. Dysfibrinogenemia:

These fibrinogen disorders are autosomal dominant and are inherited homozygously and heterozygously. They produce a qualitative disorder of fibrinogen in which an amino acid substitution produces a functionally abnormal fibrinogen molecule. Although these disorders are an academic curiosity, named

for the city in which the patient was discovered, they are infrequently associated with a bleeding tendency. A few are associated with thrombosis. Approximately 40 abnormal fibrinogens have been discovered. Because fibrin formation is affected by the abnormal fibrinogen molecule in dysfibrinogenemia, most of the normal laboratory assessments for fibrinogen will be abnormal. The PT, aPTT, TT, and reptilase time will be increased. An immunologic assay of fibrinogen that measures the antigenic level of fibrinogen is normal. The clottable assay for quantitative fibrinogen is abnormal as this assay is dependent on the proper amount and proper functioning fibrinogen (Betty. 2007).

1.2.12. Lupus anticoagulant:

The paradoxically named lupus anticoagulant (LA) is arguably the commonest coagulation abnormality predisposing to thrombosis. It is something of a misnomer as it increases the risk of thrombosis not bleeding. It is an IgG /IgM autoantibody and prolongs phospholipid dependent coagulation tests (hence the use of the term anticoagulant); bleeding is very rare despite the prolonged APTT. The LA and other antiphospholipid antibodies (APL) are found in association with arterial or venous thrombosis and/or recurrent fetal loss, the ‘antiphospholipid syndrome’, first described by Hughes in 1988 (Drew. *et al*, 2004).

1.2.13. Nature of lupus anticoagulant antibodies:

Lupus anticoagulant (LA) is antibodies that inhibit certain in vitro phospholipid dependent coagulation reactions, typically the conversion of prothrombin to thrombin (Roubey.1994).

1.2.14. Lupus anticoagulant syndrome:

Lupus anticoagulants – also known as LA or lupus inhibitors – are antibodies that attack plasma proteins in the blood .Normally , antibodies help prevent infections

by attacking foreign cells in the body, such as bacteria or viruses .In the case of the lupus anticoagulant , however , antibodies see plasma proteins as foreign bodies and threats, leading to a high risk of clotting. (Bontempo.2001).

1.2.15. Systemic Lupus Erythematosus:

Lupus anticoagulants are often found in people who have systemic lupus erythematosus. Also called SLE or lupus, this is an autoimmune disease in which a persons autoimmune system attacks the body instead of protecting it . The autoimmune system may attack different parts of the body , including the blood vessels , brain , heart and joints (shied .2007).

1.2.16. Coagulation deficiency caused by antibodies:

The lupus anticoagulant interferes with lipoprotein-dependent stages of coagulation and is usually detected by prolongation of the APTT test. This inhibitor is detected in 10% of patients with systemic lupus erythematosus (SLE) and in patients with other autoimmune diseases who frequently have antibodies to other lipid - containing antigens, e.g. cardiolipin . The antibodies is not associated with a bleeding tendency but there is an increased risk of thrombosis and ,as with other causes of thrombophilia, an association with recurrent miscarriage (Hoffbrand. *et al*, 2001).

1.2.17. Miscarriage:

1.2.17.1. Definition:

Miscarriage (abortion) is the involuntary loss or termination of pregnancy prior to 24 weeks' gestation. Other definitions include the loss of pregnancy before viability. The World Health Organization definition is 'expulsion or extraction of an embryo or fetus weighing 500 g or less from its mother' (Barry. *et al*, 2012).

1.2.17.2. Etiology:

Although the causes of miscarriage in first and second trimester appear different, there is inevitably some overlap, in addition to the occasional situation where diagnosis of a first-trimester miscarriage is delayed until the second trimester (Keith *et al*, 2012).

1.2.17.3. First - trimester miscarriage:

Evidence suggests that a significant proportion of miscarriages result from chromosomal abnormalities. It is likely that abnormal implantation has a role to play in some cases and this is an area of current research. The frequency of chromosomally abnormal tissue among first - trimester miscarriages is 50-70%, the following chromosomal abnormalities are associated with miscarriage:

- Trisomies: 68%, mainly trisomy 16, 21 and 22.
- Triploidy: 17.1%.
- Monosomy: 9.8% (XO, Turner's syndrome).

Other implicated causes of first - trimester miscarriage include the following:

- Maternal disease: antiphospholipid syndrome, diabetes, thyroid disease.
- Drugs: methotrexate, some antiepileptic drugs.
- Uterine abnormalities: the role of fibroids is uncertain but they may be implicated.
- Infection: varicella, rubella and other viral illnesses (Keith. *et al*, 2012).

1.2.17.4. Second - trimester miscarriage:

- Cervix: cervical injury from surgery, cone biopsy and large loop excision of the transformation zone.
- Infection: may occur with or without ruptured membranes. May be local to the genital tract or systemic.
- Thrombophilias.

- Uterine abnormalities: submucous fibroids and congenital distortion of the cavity (uterine septae) may be implicated.
- Chromosomal abnormalities: these too may not become apparent until the second trimester (Keith. *et al*, 2012).

1.2.18. Epidemiology:

Approximately 15% of all pregnancies that can be visualized on ultrasound end in pregnancy loss. Three or more losses affect 1 -2% of women of reproductive age and two or more losses affect around 5%. Despite extensive investigation of women with three or more miscarriages, the cause of recurrent pregnancy loss remains unknown in the majority of cases. Advancing maternal age is associated with miscarriage. Age - related miscarriage rates are as follows: 12- 19 years, 13%; 20-24 years, 11%; 25- 29 years, 12%; 30 - 34years, 15%; 35-39 years, 25%; 40-44 years, 51%; and 45 or more years, 93%. This is because with increasing maternal age there is a decline in both the number and quality of the remaining oocytes. An increasing number of previous miscarriages also adversely affects the risk of future miscarriage. A history of a live birth followed by consecutive miscarriages does not reduce the risk of further miscarriage substantially. Being both underweight and obese has been associated with recurrent miscarriage (Keith *.et al*, 2012).

1.2.19. Bleeding in early pregnancy:

Vaginal bleeding in the first trimester is a common event, occurring in up to 30% of all pregnancies, and about one-half of these will eventually miscarry by 12 weeks' gestation. More than 90% of spontaneous miscarriage occurs in the first trimester. Causes of bleeding in the first trimester include spontaneous abortion, physiological bleeding of a normal pregnancy, ectopic pregnancy, trophoblastic disease and non-obstetrical causes such as cervical lesions, e.g. polyps, ectropion or carcinoma (Barry. *et al*, 2012).

1.2.19.1. Types of miscarriage:

- Threatened - uterine bleeding without dilatation of cervix or passage of products of conception (POC). The fetus is still viable and the uterus is the expected size for dates. Only one-quarter of these will go on to miscarry.
- Inevitable - heavy bleeding with cervical dilatation, but without passage of POC. The fetus may still be alive, but miscarriage will occur.
- Incomplete - bleeding with cervical dilatation and passage of some, but not all, POC.
- Complete - bleeding which diminishes with complete passage of POC. Pain and bleeding ease, uterus returns to normal size and cervix is closed.
- Missed - fetal death, bleeding or pain; the fetus or embryo has been dead for some weeks, but no tissue has been passed. This is often not recognized until bleeding occurs, the patient complains that she feels 'less pregnant than before' and an ultrasound has been performed confirming fetal demise.
- Septic — any of the above that becomes infected, resulting in endometritis and often parametritis and peritonitis. (*Barry. et al, 2012*).

Septic Miscarriage:

Infection may complicate miscarriage once the cervix starts to dilate or instruments are introduced into the uterine cavity. (Kevin . 2003).

1.2.19.1.1. Causes:

1. Delay in evacuation of the uterus. Either the patient delays seeking advice, or the surgical evacuation has been incomplete. Infection occurs from vaginal organisms after 48 hours.
2. Trauma, either perforation or cervical tear. Healing is delayed and infection is more likely to be a peritonitis or cellulitis. Criminal abortions are, of course, particularly liable to sepsis (Kevin . 2003).

1.2.19.1.2. Infecting Organisms:

These are usually the vaginal or bowel commensals.

1. Group B haemolytic streptococcus.
2. Anaerobic streptococcus.
3. Coliform bacillus.
4. Clostridium welchii.
5. Bacteroides necrophorus.

Any of these organisms but particularly the last two may be the cause of septic shock (Kevin, 2003).

1.2.19.1.3. Miscarriage -Clinical Features:

1. Haemorrhage is usually the first sign and may be very heavy if placental separation is incomplete.
2. Pain is usually intermittent, 'like a small labour'. It ceases when the miscarriage is complete (Barry. *et al*, 2012).

1.2.20. Pathophysiology:

Up to 50% of spontaneous miscarriages are the result of a major genetic abnormality, e.g. trisomy. The remainder have been linked to other factors such as uterine abnormalities (such as fibroids or müllerian duct abnormalities), cervical incompetence (usually mid-trimester loss) (Barry. *et al*, 2012)

1.2.21. Changes in the pregnant mother:

Maternal changes start from very early on in the first trimester of the pregnancy.

Nearly all systems are affected (Maggie. *et al*, 2009).

1.2.21.1. Cardiac system:

Stroke volume and heart rate both increase resulting in cardiac output increasing by about 40% (from 4 to 6 *LI* mm). The pulse rate may increase by 10- 15 beats/minute. The increased flow across the heart valves results in an increase in

physiological flow murmurs heard on auscultation. There is a decrease in peripheral vascular resistance, resulting in a fall in blood pressure of about 10 mmHg in mean arterial pressure (MAP) in the first and second trimesters. The blood pressure tends to rise again after about 24 weeks 'gestation. The gravid uterus, especially in the later stages of pregnancy, can occlude the inferior vena cava when the mother lies flat on her back, and cause decreased return of blood to the heart, hypotension and reduced fetal flow (supine hypotension). This is why pregnant mothers are advised not to lie flat on their backs, and labour, delivery and anaesthesia are managed with the mother propped up or with a wedge that tilts her back. (Maggie *et al*, 2009).

1.2.21.2. Respiratory system:

There is increased oxygen requirement in pregnancy, which results in hyperventilation and deep breathing. Ventilation is increased by 40% through an increase in tidal volume (from 500 to 700 mL) rather than in the respiratory rate. CO₂ levels in pregnancy are lower than in the non - pregnant state; this helps the gradient between fetus and mother and favours transfer of CO₂ from fetus to mother across the placenta. Mothers may feel dyspnoeic; this may partly be because of the splinting of the diaphragm by the gravid uterus (Maggie. *et al*, 2009).

1.2.21.3. Body temperature:

Progesterone is thought to be responsible for the rise in body temperature of 0.5-1.0 °C during pregnancy. An increased metabolic rate and peripheral vasodilatation also result in a warm skin and may cause palmar erythema (Maggie. *et al*, 2009).

1.2.21.4. Blood and plasma volume:

Blood volume increases by approximately 1200 mL (40%), while red cell mass increases by 250 - 400 mL (about 25%). This results in a relative haemodilution

and anaemia, with a drop in haematocrit and haemoglobin levels. There is increased blood flow to a number of systems including the uterus, skin and the kidneys. There is an increase in the glomerular filtration rate (GFR) from 140 to 170 mL/minute. This results in lower serum urea and creatinine levels. These needs to be borne in mind when interpreting blood results in pregnancy, as the normal range quoted for the non - pregnant state may not be relevant, and an abnormal result may be missed. There is an increase in extravascular fluid because of lower plasma colloid osmotic pressure (albumin levels are lower in pregnancy), and this contributes to the peripheral odema that may be seen with normal pregnancy. Symptoms of carpal tunnel syndrome of tingling of the fingers may be caused by compression of the median nerve under the flexor retinaculum because of increased extracellular fluid. The blood may show a mild leucocytosis in pregnancy (9- 11 10⁹ /L compared to 4- 11 10⁹ / L) and an increase in erythrocyte sedimentation rate. Serum iron is lower and iron requirements are increased because of demands from the fetus, red cell increase and blood loss at delivery. There is no consensus as to whether routine iron supplementation is indicated in pregnancy but evidence suggests that a higher iron level following supplementation does not improve maternal or neonatal outcomes. Iron supplementation is advised at haemoglobin levels below 10.5 gm/ dL (Maggie. *et al*, 2009).

1.2.21.5. Metabolic changes:

Metabolic rate is increased to accommodate increased energy demands from the fetus and for the maternal changes. Pregnancy is also a diabetogenic state. Glucose levels are increased as placental hormones are insulin antagonists and there is an increased insulin resistance in pregnancy. If sufficient insulin cannot be produced to counter this resistance, gestational diabetes may occur. This physiological

change also explains why insulin requirements in known insulin - dependent diabetic mothers increases during pregnancy (Maggie. *et al*, 2009).

1.2.21.6. Coagulation profile:

Pregnancy is a hypercoagulable state. This is a protective mechanism for the mother to be able to minimize blood loss after delivery of the placenta. There is an increase in fibrinogen, factor VIII and von Willebrand factor, and a decrease in anticoagulants such as antithrombin III. There is also an increase in fibrinolytic activity. While this hypercoagulable state is protective against bleeding, it results in an increased risk of thrombosis during pregnancy, especially when there are other risk factors present (e.g. increasing age, smoking, high BMI, operative delivery, prolonged immobilization) (Maggie. *et al*, 2009).

1.2.21.7. Maternal weight gain:

This is around 11-12 kg through the pregnancy. Much of this increase is caused by the extravascular retention of fluid (approximately 8.5 L), increase in blood volume, growth of the uterus, breasts and body fat. The fetus (around 3.5 kg), placenta and liquor (about 0.7 -0.8 kg each) account for about 4.5—5 kg of the weight gain (Maggie. *et al*, 2009)

1.2.22. Previous studies:

In their study Nine hundred and twenty - five (925) women with recurrent fetal loss (RFL) were recruited consecutively from a special outpatient RFL clinic ,King Khalid University Hospital, Riyadh. They found that when comparing the prevalence of LA between women with normal pregnancy or blood donors and those with RFL, dRVVT is the only test that showed a markedly higher prevalence of LA among women with RFL (10.9 %) than normal pregnancy (5.6 %, $p < 0.0279$) or healthy blood donors (1.2 % $p < 0.001$). For RFL patients, the prevalence of LA was slightly higher for dRVVT (10.9 %) when compared to the

either the APTT (10.2%) or the KCT (10.5 %). The values for any of the tests being remarkably higher than in blood donors (Al-Mishari. *et al*, 2004).

In Nigeria in their study they had used a combination of activated partial thromboplastin time (aPTT) and Kaolin clotting time (KCT) to determine the presence of the antibody in 125 healthy individuals which included pregnant women. Sex (4.8 %) and four (3.2 %) of the subjects had elevated a PTT and a KCT ratio 1.2 respectively .The tests showed a high prevalence of the lupus anticoagulant in the multiparous group than the other groups while there is poucity of the anticoagulant in the pregnant women who are not at risk. They suggest the use of both aPTT and KCT for the screening of patients in whom the antiphospholipid syndrome is suspected (Alkinmoladun. *et al*, 2007). In their study two hundred women with recurrent mid- trimester abortion were randomly enrolled from a main referral center in Baghdad —Iraq fifty three (26.5 %) had one or both anticardiolipin antibodies present, while 27 (13.5 %) were positive for lupus anticoagulant. The KCT and KCT index appeared to be the most sensitive tests, while the KCT index and APTT were the most specific for lupus anticoagulant patients with antiphospholipid antibodies had higher rates of history of thrombosis, thrombocytopenia and family history of recurrent abortion $p=0.0009$, 0.0056 and 0.0003 respectively). (Amel .*et al*, 2012).This study was done to detect lupus anticoagulant in fifty patients with three or more recurrent pregnancy losses. Twenty women with normal obstetric history were included as controls .The tests used for detecting the lupus anticoagulant were the dPT, dAPTT ,dRVVT ,KCT, and PNP. The study showed that two of the patients (4 %) had lupus anticoagulant as suggested by prolonged dAPTT, which was not corrected by mixing with normal plasma and was shortened by PNP. The study also showed that although the mean of dAPTT, of the LA positive patients was significantly elevated compared to the LA negative patients ($p < 0.05$), the mean of dPT ,

dRVVT and KCT of LA positive patients compared to LA negative patients showed no statistical significant difference ($p > 0.005$) (Tagreed .2006).

1.2.23. Rationale:

The aim of this study is to investigate lupus anticoagulant among Sudanese women with habitual abortion, Furthermore, to evaluate the titer of circulating anticardiolipin antibodies, which have been reported as being strongly associated with lupus anticoagulant. There is no well-established screening test for lupus anticoagulant that can be used for screening of high risk group, and hence help in reducing LA morbidity. Identification of associated assay marker can play an important role in identification of patients that should go for confirmatory test and thus may limit the use of expensive test.

1.2.24. Objectives of the study:

1.2.24.1. General objective:

To investigate lupus anticoagulant among Sudanese women with habitual abortion. In an attempt to find out the role of LA in cases habitual abortion.

1.2.24.2. Specific objective:

- 1- To estimate lupus anticoagulant (LA), anticardiolipin antibodies (ACA) and fibrinogen level.
- 2- To evaluate anticardiolipin antibodies (ACA) IgG ,in patients suspected to have LA.
- 3-To measure the frequencies and association of lupus anticoagulation (LA) and anticardiolipin antibodies (ACA) in women with habitual abortion and compared with the control group.

Chapter Two

2. Materials and Methods

2.1. Study design:

This was a descriptive cross sectional study in which survey was conducted among Sudanese women with habitual abortion. The all information collection by questionnaire during December 2013 to July 2015.

2.2. Study area:

The study was conducted in Omdurman Maternity Hospital ,Al Saudi Hospital and Bahary Teaching Hospital .

2.3. Study population (patients and controls):

Healthy pregnant women without history of abortion were chosen as the control group and women with history of recurrent first and second trimester fetal loss as the study group.

2.4. Sample size:

The study was performed on one hundred and forty (140) women with history of three or more recurrent abortion in first and second trimester , and on sixty (60) healthy pregnant women without history of abortion as the control .

All at age from 17 – 45 years.

2.5. Inclusion criteria:

Women with history of (three or more) recurrent abortion in first and second trimester.

2.6. Exclusion criteria:

1-Non Sudanese women

2-Presence of other disease

3-Women with single or twice abortion

2.7. Data collection:

The data were collected using a questionnaire and LA investigation were conducted.

2.8. Sampling preparation:

2.8.1. Sampling:

The plasma (4.5 ml) was obtained from whole blood anticoagulated with 0.5 ml (3.2%) Tri-sodium citrate.

2.8.2. Sample preparation:

The whole blood specimen was centrifuged at 2000 X g for 15 minutes. Immediately, the plasma was separated from the red blood cell using a plastic pipette and was placed it in a plastic test tube and was frozen at -70 c and was stored until performing the tests.

2.9. Ethical consideration:

Selected individuals was informed consent with detailed the objectives of the study and its importance in the future, and informed about the procedure and the amount of blood sample that was withdrawn , and to inform them that the results was confidentially

2.10. Procedure:

2.10.1 Lupus Anticoagulants (LA):

This test is used for the screening and confirmation of lupus Anticoagulant (LA)

2.10.1.1. Principle:

Russell's viper venom directly activates factor X in the presence of phospholipid and calcium ions, bypassing factor VII of the extrinsic pathway and the contact and antihemophilic factors of the intrinsic pathway. In normal plasma in the absence of lupus of anticoagulants. Factor X is directly activated by Russell's Viper venom,

which in presence of phospholipid and calcium ion Leads to clot formation. In patients with LA, autoantibodies bind the epitopes of reagent phospholipids thereby preventing the activation of prothrombinase complex . This results in a prolongation of clotting time with LA reagent.

2.10.1.2. Reagents:

Fortress LA Kit comprises of a 3 – reagent set for screening and confirmation of lupus anticoagulants. Reagent 1(R1) and Reagent 2(R2) are lyophilised preparations containing Russell's Viper Venom enriched with phospholipid at different concentrations, sufficient for performing 10 assays. Both R1 and R2 reagents contain 0.01% thimerosal as preservative.

Calcium Chloride (Reagent 3 (R3), 0.025M) contains heparin neutralizing substance, making the reagent system insensitive to the Presence of heparin up to 0.4 U/ml Calcium Chloride reagent was intended for use with R1 and R2 reagents .

2.10.1.3. Storage and stability:

1. The reagents stored at 2-8°C.
2. The reagents were stable up until the expiry dates shown on the reagent vials.
3. Reconstituted reagents were stable up to 5days when stored at 2-8C. the reagent contamination was avoided .

2.10.1.4. Reagent preparation:

The reagents were brought to room temperature (about 25°C) prior to reconstitution.

1. 1 ml of distilled water was added to the lyophilised R1 (LA screen) and R2 (LA confirm) reagent.
2. Gently mixed to dissolve and kept for 15-20 minutes at room temperature. Mixed again gently to ensure complete re–suspension of the lyophilized material.
3. The reagent was mixed well before withdrawing material every time for testing.

2.10.1.5. Test procedure:

1. The reagents were brought to room temperature (about 25°C) before prewarming at 37°C for testing.
2. The required quantity of calcium chloride was aspirated in a test tube . Incubated at 37°C for 10 minutes.
3. 0.1 ml of R1 (LA screen) was placed in a clean and dry test tube, mixed well before withdrawing material for testing.
4. 0.1 ml of platelet poor plasma was added ,shacked gently to mix the contents and incubated for 1-2 minutes at 37 °C .
5. 0.1 ml of calcium chloride (prewar med at 37°C for 10 minutes) was added and simultaneously the stopwatch was started.
6. The stopwatch should be stopped as soon as the clot formation begins. The time in seconds was recorded. This is the screen time for the plasma specimen. If screen time was less than 45 seconds, it indicates absence of LA and there is no need to perform confirmatory test.
7. When the screen time was more than 45 seconds the test had been repeated procedure for the sample using R2(LA confirm) reagent.

Repeat the steps 2 – 6 for testing sample plasma specimen using R2 (LA confirm) reagent.

2.10.1.6. Normal value:

1. The normal values for screen time is 28 – 45 seconds.
2. The normal values for confirm time is 28 – 40 seconds.

2.10.2. Fibrinogen:

2.10.2.1. Principle:

The determination of fibrinogen with thrombin clotting time is based on the method originally described by Clauss ; in the presence of an excess of thrombin, fibrinogen is transformed into fibrin and clot formation time is inversely proportional to the concentration of fibrinogen in the sample plasma (Clauss .1957).

2.10.2.2. Method of Fibrinogen estimation:

A calibration curve was prepared each time the batch of thrombin reagent is changed or there is a drift in control results ; this was used to calculate the results of unknown plasma samples.

a dilutions was made of the calibration plasma in veronal buffer to give a range of fibrinogen concentrations (i.e., 1 in 5 , 1 in 10 , 1 in 20 ,and 1 in 40). Part (0.2 ml) of each dilution is warmed to 37°C , 0.1 ml of thrombin solution is added ,and the clotting time is measured .Each test should be performed in duplicate. Plot the clotting time in seconds against the fibrinogen concentration in g/l on log/log graph paper. The 1 in 10 concentration is considered to be 100%, and there should be a straight line connection between clotting times of 5 and 50 sec. Make a 1 in 10 dilution of each patients sample and clot 0.2 ml of the dilution with o.1 ml of thrombin. The fibrinogen level can be read directly off the graph if the clotting time is between 5 and 50 sec. However, outside this time range a different assay dilution and mathematic correction of the result will be required (i.e,if the fibrinogen level is low and a 1 in 5 dilution is required , divide answer by 2 and for a 1 in 20 dilution multiply answer by 2).(Dacie. *et al* ,2001).

2.10.2.3. Reference Range:

180 – 450 mg fibrinogen /100ml.

2.10.3. ELISA: for the Quantitative Determination of Anti – Cardiolipin Antibodies (IgG)

2.10.3.1 Principle:

The test is based on the immobilisation of cardiolipin and purified human beta-2-glycoprotein 1 to the solid phase of microtiter strips and subsequent binding of anti-cardiolipin antibodies from patient serum.

The bound antibodies are detected with a peroxidase – labelled secondary antibody that is directed against human IgG. After addition of substrate solution, a colour appears which intensity is proportional to the concentration and / or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow.

2.10.3.2. Contents:

Reagent of anticardiolipin antibodies (IgG) (contents of the kit):

- 12 microtiter strips (in 1 strip holder) 8 – well snap – off strips, ready for use, Coated with cardiolipin and purified human beta 2 – glycoprotein 1
- 1.5 ml calibrators IgG (white cap). Human serum, inked according to concentration. , ready for use, Anti- cardiolipin level: 31.25u/ml (1), 62.5 u/ml (2), 123 u/ml (3), 250 u/ml (4), 500 u/ml (5)
- 1.5 ml negative control serum (green cap) , human , ready for use
- 1.5 ml positive control serum (red cap), human, ready for use
- 50 ml washing buffer (black cap) concentrate (20x) for 1 TRIS buffer PH6.9+0.2
- 100ml dilution buffer (blue cap) ready for use Phosphate buffer PH7.3+0.2
- 15 ml conjugate solution (white cap) anti – human -IgG HRP conjugate, ready for use.

- 15 ml TMB solution (black cap) ready for use PH 3.7+0.2 Colourless to bluish

3.3.5.5- tetramethylbenzidin 1.2 mmol/l

Hydrogen peroxide 3 mmol/l

- 15 ml stop solution (red cap) sulphuric acid , ready for use 0.5 mol/l

1. Adhesive strip.

2.10.3.3. Stability:

The reagents were stable up to the stated expiry dates on the individual labels when stored at 2-8°C.

2.10.3.4. Reagent preparation:

Washing Buffer Solution WASH

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part WASH 20x with 19 parts distilled water WASH is stable for 6 weeks stored at 2...8°C.

2.10.3.5. Specimen:

Allow the samples to reach room temperature (30min) Dilute samples 1:101 with DIL (add 10ul sample to 1mL DIL).

2.10.3.6. Procedure:

1. 100µl of diluted patient sample, calibrators, positive and negative was pipetted into microtiter.
2. for blank dilution buffer was used instead of sample dilution, microtiter was sealed with adhesive strip.
3. Incubated for 1 hour at RT.
4. The solution from microtiter was discarded. Washed microtiter 3 times using 300 ml washing buffer per well.
5. The residues was discarded Washed and knocked out on an absorbent paper or cloth.

6. 100µl conjugate solution was pipetted and sealed microtiter with adhesive strip.
7. Incubated for 30 minutes at RT.
8. The solution was discarded from microtiter . Washed microtiter 3 times using 300 µl washing buffer per well.
9. The residues were discarded Washed and knocked out on an absorbent paper or cloth.
10. 100 µl of SUB solution was pipetted and incubated for 10 min.
11. 100µl of stop solution was added per well.
12. The absorbance values were read at 450 nm within the next 10 min, after stopping Bi-chromatic measurement with a reference wavelength at 620 – 690 nm recommended.

2.11. Data analysis:

The collected data was analyzed to obtain the mean , standard deviation, frequencies and the probability (p- value) between patients and control using Statistical package of Social Sciences (SPSS version 21) computer program .

Chapter Three

3. Results

The age group most affected is between 28-33 years comprising (27.9%) of the study group, and lowest affected age group is between 40-45 years comprising (5.0%) of the study group as show in table (1)

Also the majority of patients were came from South Kurdfan 23 (16.4%) and the least were from Blue Nile & Red Sea (0.0%) show in table (2). The mean of LA was significantly increased in study group compared with control group (p,value 0.00). The mean of ACA was significantly increased in study group compared with control group (p,value 0.00). Also the mean of fibrinogen level show insignificant variation between two groups (p,value 0.449) as show in table (3).

There was significant correlation between the presence of lupus anticoagulants (LA) and the age ($p= 0.03$) in women with habitual abortion. The mean of LA in women with habitual abortion it is seen more in the age group between 34-39 years as shown in table (4).

Figure (4) show the frequency of abortion, the most of study group comprising 78.6%. The lowest was comprised 0.7%.

Characterize	<i>N(%)</i>			
Ages/years	<i>Number of Patients and %</i>		<i>Control and %</i>	
16-21	19	(14)	17	(28)
22-27	37	(26)	16	(27)
28-33	39	(28)	20	(33)
34-39	38	(27)	5	(8)
40-45	7	(5)	2	(3)
Total	140	100	60	100

Table 3.1: Distribution of study population according to age group

Sudan States	<i>Patients %</i>		<i>Control %</i>	
White Nile	12	(9)	3	(5)
Khartoum	13	(9)	5	(8)
Al Qadarif	5	(4)	3	(5)
Northern	17	(12)	5	(8)
River Nile	3	(2)	4	(7)
Sennar	8	(6)	5	(8)
North Darfur	8	(6)	6	(10)
West Darfur	6	(4)	2	(3)
South Darfur	2	(1)	0	(0)
East Darfur	4	(3)	0	(0)
South Kurdfan	23	(16)	4	(7)
North Kurdfan	16	(11)	6	(10)
West Kurdfan	2	(1)	0	(0)
Central Darfur	1	(0.7)	0	(0)
Kassala	5	(4)	2	(3)
Blue Nile	0	(0)	3	(5)
Red Sea	0	(0)	3	(5)
Al jazirah	10	(7)	9	(15)
Total	140	100	60	100

Table 3.2: Sudanese states distribution of patients and controls

Parameters	Mean \pm SD		P.value
	Patients	Control	
Lupus Antibodies(LA)	61.4 \pm 21.6	36.422 \pm 4.6	0.00
AntiCardiolipin Antibodies (ACA)	242.5 \pm 124.3	40.4 \pm 4..51	0.00
Fibrinogen	265.28 \pm 118.9	255.1 \pm 68.5	0.449

P. value < 0.05 is significant

Table 3.3: Mean of LA, ACA and Fibrinogen levels compared to control group.

Age groups/years	Mean \pm SD		
	Lupus antibodies screening/ seconds	Anticardiolipin antibodies U /ml	Fibrinogen mg/ 100mL
16 – 21	60.3 \pm 10.3	226.8 \pm 108.9	260.2 \pm 130.9
22 – 27	57.0 \pm 15.0	241.3 \pm 121.4	278.9 \pm 109.0
28 – 33	61.4 \pm 26.4	259.9 \pm 138.9	275.4 \pm 134.6
34 – 39	66.2 \pm 25.6	239.5 \pm 121.1	236.9 \pm 96.4
40 – 45	61.5 \pm 20.2	210.9 \pm 131.5	304.9 \pm 156.3

Table 3.4: Age related incidence distribution compared to LA, ACA and fibrinogen levels among the study groups

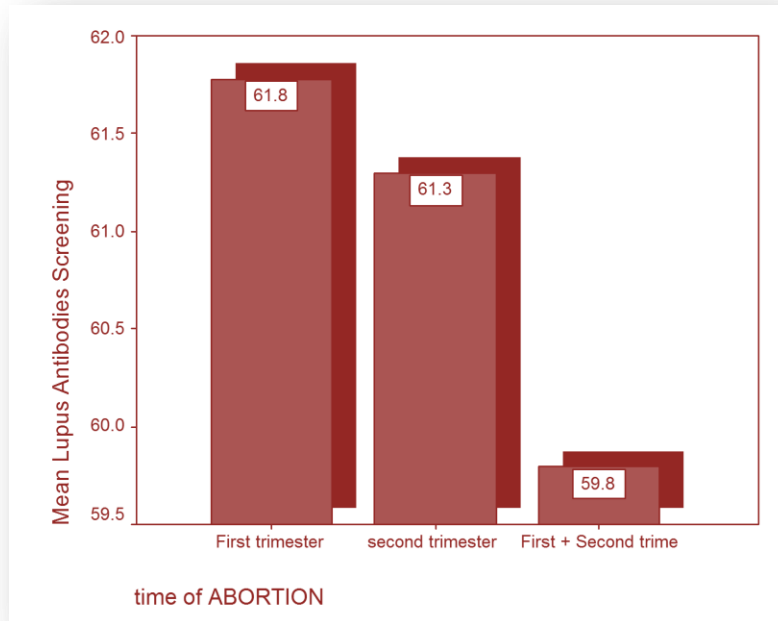


Figure 3.1: The mean LA level compared to the time of abortion among the study groups

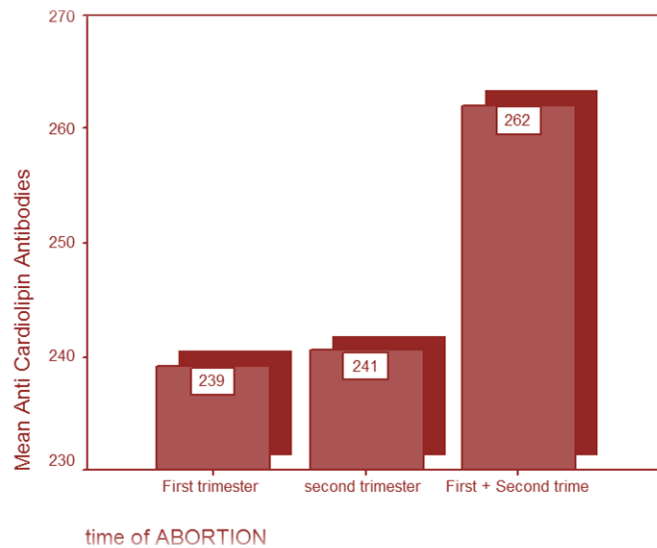


Figure 3.2: The mean ACA level compared to the time of abortion among the study groups

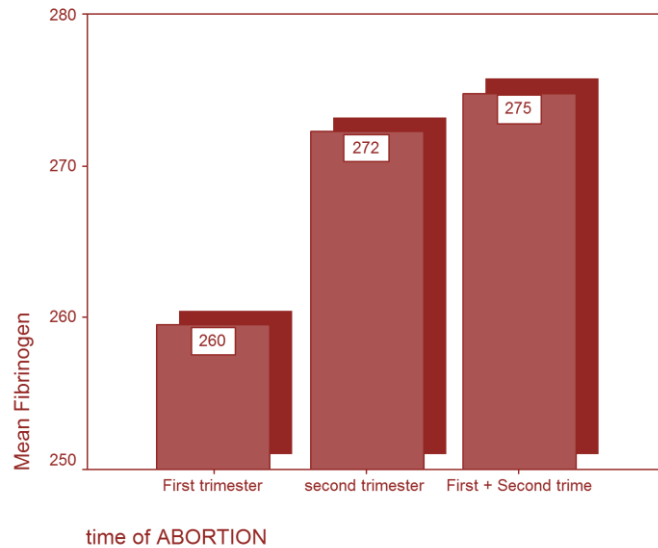


Figure 3.3: The mean fibrinogen level compared to the time of abortion among the study groups

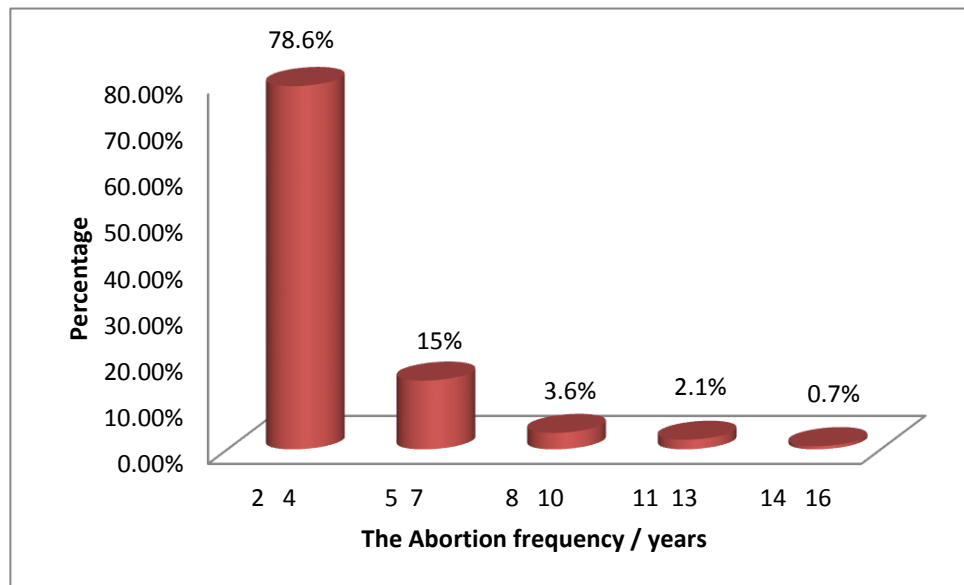


Figure 3.4: The frequency of abortion among the study group, n = 140.

Chapter Four

Discussion, Conclusion & Recommendations

4.1. Discussion:

This is a descriptive cross sectional study which conducted in Khartoum state during the period of 2013 to 2015 to investigate lupus anticoagulant among Sudanese women with habitual abortion.

The mean of LA was significantly increased in study group compared with control group (p,value 0.00). The mean of ACA was significantly increased in study group compared with control group (p,value 0.00). In the present study there was insignificant difference between the mean level of fibrinogen in study group of patients and control group (p,value 0.449).

This study is added to some previous studies in Sudan which shows statistically significant difference of lupus anticoagulant in recurrent miscarriage .Also the data in this study extend and confirm the data obtained in other studies in Sudan carried out by (Tagreed . 2006 & Tagwa. *et al*, 2011) and other countries .

The present study showed that 26.4 % of patients with habitual abortion had a positive result for LA, while 34.3 % had positive for ACA. The finding is consistent with study, had been done in Iraq, India and Nigeria. Although it is considered a lowest percentage but it was high of LA among Sudanese women with habitual abortion when compared to other studies conducted in Sudan and other countries. In Iraq study the frequencies of LA and ACA in sera of women with recurrent miscarriage were (LA 13.5 %, ACA 26.5 %) carried out by (Amel. *et al*, 2012).

In Indian the frequencies of ACA in sera of women with recurrent miscarriage was 40% carried out by (Velaguthaprabhu. *et al*,2005).Another report by

(Alkinmoladin *et al*,2007) from Nigeria shown that the frequencies of LA were 4.8 %. The prevalence of ACA in patients with habitual abortion , as determined in this study is 34.3% and this is lowest than the rate reported by (Velaguthaprabbu. *et al*,2005) on the other hand, a much higher frequency of 40.3 % was reported from India.

Also the result of the current study were confirmed by (Tagreed . 2006), the study showed that 4% of women with recurrent had LA, other result of study in Sudan done by (Tagwa. *et al*, 2011), agreed with results, this study which found 20 % of women with recurrent miscarriage had LA. While 20% had ACA. In addition, other study in Sudan was found the frequencies of LA were 20% and ACA was 20% from women with recurrent miscarriage this study done by (Ahmed .*et al*, 2013).

The variations noted in the rates of LA and ACA is related to patient selection, various pre-analytical variable, differing sensitivities of tests, isotypes tests and the population studies (Lakos .*et al*, 2012). The use of variable types and numbers of tests with variable sensitivities and methodologies, differences in the numbers of cases included and the criteria of patient selection (Geis .*et al*, 2001 &Oshiro .*et al*,1996). The current study showed that most affected age was in range between 28-33 years comprising 27.9% of the study group, and lowest effected age was between 40-45 years comprising 5.0% of the study group as show in table (1). The majority of patients were came from South Kurdfan 23(16.4%) and the least were from Blue Nile & Red Sea 0 (0.0%) show in table (2) the finding is consistent with study done in South Kurdufan carried out by (Mahmoud . 2009) the study show the incidence of abortion was 10.1 % .

4.2. Conclusion:

In conclusion, the presence of LA and ACA in women with habitual abortion were significantly associated with habitual abortion and the age. LA is a significant cause of recurrent fetal loss in Sudanese women. The dRVVT is the best screening test for LA. The LA was not detected in healthy pregnant Sudanese women without history of abortion.

4.3. Recommendations:

- 1-Women with habitual abortion should be screened for LA using dRVVT test as screening coagulation test , ACA IgG test using ELISA method.
- 2-Women with habitual abortion should be routinely screened for coagulation tests.
- 3- It is there for advisable to routinely use the test (dRVVT) when screening for LA.
- 4- Women with habitual abortion should be informed about risk factors of coagulation during pregnancy.

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Appendices

Appendix (1) Questionnaire
Sudan University of Science & Technology
Collage of Graduate Studies
Questionnaire on :
Detection of lupus Anticoagulant Antibodies among Sudanese Women
with Habitual Abortion

*Serial number:

* Age:

*State:

*Trimester:

*Family history of disease: Yes ☐ No ☐

If yes the disease is

.....

*Abortion: Yes ☐ No ☐

*No of abortion

1. First trimester

2. Second trimester

3. Third trimester

Others

LABORATORY INVESTIGATION

LAsec

Fibrinogen Levelmg/100ml

ACAu/ml

Date \ \

sig.....