Screening of Lupus Anticoagulant in Women with Recurrent Miscarriage Attending Wad Medani Obstetrics and Gynecology Teaching Hospital Gezira State, Sudan

Zeinab Mohamed Modwy Fadl Alla

(B.Sc, honor degree in Haematology, University of Gezira 2009)

A Dissertation

Submitted to the University of Gezira in Partial Fulfillment for the Requirements for the Award of the Degree of Master of Science in Haematology and Immunohaematology

Department of Haematology

Faculty of Medical Laboratory Sciences

University of Gezira

May 2015
Screening of Lupus Anticoagulant in Women with Recurrent Miscarriage Attending Wad Medani Obstetrics and Gynecology Teaching Hospital Gezira State, Sudan

Zeinab Mohamed Modwy Fadl Alla

Supervision Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Albadawi Abdelbagi Talha</td>
<td>Main supervisor</td>
<td></td>
</tr>
<tr>
<td>Dr. Abdel Rahim DafaAlla Haggaz</td>
<td>Co supervisor</td>
<td></td>
</tr>
</tbody>
</table>

Date: May/ 2015
Screening of Lupus Anticoagulant in Women with Recurrent Miscarriage Attending Wad Medani Obstetrics and Gynecology Teaching Hospital Gezira State, Sudan

Zeinab Mohamed Modwy Fadl Alla

Examination Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Albadawi Abdelbagi Talha</td>
<td>Chairman</td>
<td>...........</td>
</tr>
<tr>
<td>Dr. Sara Abdelmonaim Abdelrazig</td>
<td>External Examine</td>
<td>.........</td>
</tr>
<tr>
<td>Dr. Rania Mahgoub Seed Ahmed</td>
<td>Internal Examiner</td>
<td>.........</td>
</tr>
</tbody>
</table>

Date of examination: / 5 / 2015
**Dedication**

I would like to dedicate this work to:

*The source of compassionate love*

*My beloved mother*

*The symbol of giving faithfulness*

*My compassionate father*

*My wonderful brothers and sisters for their valuable encouragement.*

*Each and every one of my colleagues and friends who participated in bringing this*

*Project to the happy end.*
Acknowledgment

I would like first and most to thank almighty God for the blessings and power that made my project a reality. I would like to express my gratitude to my supervisors: Dr. Albadawi Abdelbagi Talha, associated professor of medical parasitology, Faculty of Medical Laboratory Science, University Of Gezira and Dr. Abd Elrahim Haggaz, general director of Wad Medani Obstetrics and Gynecology Teaching Hospital of, associate professor of obstetrics and gynecology University Of Gezira, I wish to give my warmest thanks for all the members of Faculty of Medical Laboratory Science in University of Gezira.

My thanks should be extended to the laboratory teams of Wad Medani Obstetrics and Gynecology Teaching Hospital for their encouragement and supporting.

It is impossible to convey, in a couple of sentences, my gratitude to many people for helping me to learn and who cooperation made this work possible.
Screening of Lupus Anticoagulant in Women with Recurrent Miscarriage Attending Wad Medani Obstetrics and Gynecology Teaching Hospital Gezira State, Sudan

Zeinab Mohamed Modwy Fadl Alla
MSc. Haematology and immunohaematology May, 2015
Faculty of Medical Laboratory Sciences
University of Gezira

Abstract

Recurrent miscarriage (RM) defined as three or more consecutive pregnancy losses. It affects about 5-15% of all pregnancies worldwide. Recurrent miscarriage is a critical problem in which many factors play a crucial role such as Lupus anticoagulants (LA) which are Immunoglobulins usually IgG, IgM or both directed against plasma proteins such as prothrombin or annexin V that are bound to phospholipids. This study was aimed to screening of lupus anticoagulant in women with unexplained recurrent miscarriages in Wad Medani Teaching Hospital of Obstetrics and Gynecology also the study aimed to determine the association between age of women with recurrent miscarriage, number of miscarriage, and family history of recurrent miscarriage, pregnancy and age of fetal loss with positive cases of lupus anticoagulant (LA). This study was a descriptive cross sectional study conducted from January - April 2015. A total of 50 women with three or more recurrent miscarriage were screened for the presence of lupus anticoagulant (LA). The study was found the occurrence of lupus anticoagulant (LA) was 3/50 (6%) in women with recurrent unexplained miscarriage in Wad Medani Teaching Hospital of Obstetrics and Gynecology. The study was found that 1/50 (33.3%) of positive cases for (LA) in the age between (20 – 30), were pregnant and had three miscarriages, and (66.7%) of positive cases their age between (31- 40) years, were non pregnant and had six miscarriages. All positive cases 3/50 (100%) for lupus anticoagulant have a family history of recurrent miscarriage and their age of fetal loss between (1 – 13) weeks. The study was reported there was a statistical significant association between family history of recurrent miscarriage (p value=0.04), age of fetal loss (p value=0.03), and positively of lupus anticoagulant and there was no a statistical significant association between age of women with recurrent miscarriage, pregnancies,
number of miscarriages and positively of lupus anticoagulant. the researcher recommended to do the screening of lupus anticoagulant as a part of routine investigation for women with unexplained recurrent miscarriage.
الإجهاض المتكرر يعرف بأنه فقدان الحمل ثلاثة مرات متتالية أو أكثر. يؤثر الإجهاض المتكرر على حوالي 5 - 15٪ من جميع حالات الحمل في جميع أنحاء العالم. الإجهاض هو مشكلة خطيرة فيها العديد من العوامل التي تلعب دورا حاسما مثل مضادات تخثر داء الذئبة التي هي غлюбولينات مناعية عادة الغлюбولين المناعي (اي جي ام)، ( اي جي جي) أو كليهما موجهة ضد بروتينات البلازما مثل البروتومين و انكسين في التي لا بد أن تكون مرتبطه بالدهون الفوسفاتية. تهدف هذه الدراسة إلى استقصاء مضاد تخثر داء الذئبة في النساء ذوات حالات الإجهاض المتكررة غير المبررة في مستشفى ود مدني التعليمي لأمراض النساء والتوليد. أيضا هدفت الدراسة لإيجاد علاقة بين عمر المرأة ذات الإجهاض المتكرر، وعدد مرات الإجهاض، التاريخ الأسري من الإجهاض المتكرر والحمل وعمر فقدان الجنين باستخدام الحالات الإيجابية لتخثر داء الذئبة. وكانت هذه الدراسة دراسة وصفية مقطعية أجريت في الفترة من يناير حتى أبريل 2015. تم فحص مجموعه 50 امرأة ذات ثلاثة مرات أو أكثر من الإجهاض المتكطرة وذلك باستخدام استقصاء مضادات تخثر داء الذئبة. وجدت الدراسة أن حدوث مضاد تخثر داء الذئبة هو 3/50 (6٪) في النساء ذات الإجهاض المتكررة غير المبررة في مستشفى ود مدني التعليمي لأمراض النساء والتوليد. وجدت الدراسة أن 1/50 (33.3%) من الحالات الإيجابية لمضاد تخثر داء الذئبة اعمارهن بين (20 – 30) سنة، كن حاليات ولهن ثلاثه اجهاضات و (66.7%) من الحالات الإيجابية اعمارهن بين (31 – 40) سنة، غير حاليات ولهن سته اجهاضات. كل الحالة الإيجابية 3/50 (100%) لمضاد تخثر داء الذئبة في تاريخ اسري للإجهاض المتكرر وعمر فقدان اجتهن بين (1 – 13) أسبوع. أفادت الدراسة بوجود ارتباط ذو دلالة إحصائية بين التاريخ الإساري من الإجهاض المتكرر ( القيمة الحصانية = 0.04)، عمر فقدان الجنين ( القيمة الحصانية = 0.03) وإيجابية مضاد تخثر داء الذئبة وعدم وجود ارتباط ذو دلالة إحصائية بين عمر المرأة ذات الإجهاض المتكرر، الحلم، عدد مرات الإجهاض المتكرر وإيجابية مضاد تخثر داء الذئبة. أوصت الدراسة بأن الكشف عن مضاد تخثر داء الذئبة ينبغي أن يكون جزءا من الفحص الروتيني للنساء ذوات الإجهاض المتكرر غير المبرر.
# List of contents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>II</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>III</td>
</tr>
<tr>
<td>Abstract (English)</td>
<td>IV</td>
</tr>
<tr>
<td>Abstract (Arabic)</td>
<td>V</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>VI</td>
</tr>
<tr>
<td>List of abbreviation</td>
<td>IX</td>
</tr>
<tr>
<td>List of tables</td>
<td>XI</td>
</tr>
<tr>
<td>List of figure</td>
<td>XII</td>
</tr>
<tr>
<td><strong>Chapter 1: Introduction</strong></td>
<td></td>
</tr>
<tr>
<td>1.1. Overview</td>
<td>1</td>
</tr>
<tr>
<td>1.2. Justification</td>
<td>2</td>
</tr>
<tr>
<td>1.3. Objectives</td>
<td>3</td>
</tr>
<tr>
<td>1.3.1 General objective</td>
<td>3</td>
</tr>
<tr>
<td>1.3.2. Specific objectives</td>
<td>3</td>
</tr>
<tr>
<td><strong>Chapter 2: Literature Review</strong></td>
<td></td>
</tr>
<tr>
<td>2.1. Pregnancies</td>
<td>4</td>
</tr>
<tr>
<td>2.2. Normal haemostatis</td>
<td>4</td>
</tr>
<tr>
<td>2.2.1 Blood vessels</td>
<td>4</td>
</tr>
<tr>
<td>2.2.2 Platelets</td>
<td>5</td>
</tr>
<tr>
<td>2.2.3 Coagulation cascade</td>
<td>5</td>
</tr>
<tr>
<td>2.2.4 Coagulation inhibitors</td>
<td>6</td>
</tr>
<tr>
<td>2.3. Pregnancy and haemostasis</td>
<td>7</td>
</tr>
<tr>
<td>2.4. Recurrent miscarriage</td>
<td>7</td>
</tr>
<tr>
<td>2.4.1. Genetic etiologies</td>
<td>8</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>2.4.2. Anatomic etiologies</td>
<td>8</td>
</tr>
<tr>
<td>2.4.3. Endocrine etiologies</td>
<td>8</td>
</tr>
<tr>
<td>2.4.4. Environmental etiologies</td>
<td>8</td>
</tr>
<tr>
<td>2.4.5. Infectious etiologies</td>
<td>8</td>
</tr>
<tr>
<td>2.4.6. Immunologic etiologies</td>
<td>8</td>
</tr>
<tr>
<td>2.4.7. Thrombotic etiologies</td>
<td>9</td>
</tr>
<tr>
<td>2.4.7.1. Inherited thrombophilia</td>
<td>9</td>
</tr>
<tr>
<td>2.4.7.2. Acquired thrombophilia</td>
<td>9</td>
</tr>
<tr>
<td>2.5. Antiphospholipid syndrome</td>
<td>10</td>
</tr>
<tr>
<td>2.6. Lupus anticoagulant (LA)</td>
<td>10</td>
</tr>
<tr>
<td>2.6.1. Pathophysiology of LA</td>
<td>11</td>
</tr>
</tbody>
</table>

**Chapter 3: Materials and Methodology**

<table>
<thead>
<tr>
<th>3.1. Study design</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2. Study area</td>
<td>12</td>
</tr>
<tr>
<td>3.3. Study population</td>
<td>12</td>
</tr>
<tr>
<td>3.4. Study period</td>
<td>12</td>
</tr>
<tr>
<td>3.5. Sampling and sample size</td>
<td>12</td>
</tr>
<tr>
<td>3.6. Inclusion criteria</td>
<td>12</td>
</tr>
<tr>
<td>3.7. Exclusion criteria</td>
<td>12</td>
</tr>
<tr>
<td>3.8. Ethical consideration</td>
<td>13</td>
</tr>
<tr>
<td>3.9. Data collection</td>
<td>13</td>
</tr>
<tr>
<td>3.9.1. Questionnaire interview</td>
<td>13</td>
</tr>
<tr>
<td>3.9.2. Specimen collection</td>
<td>13</td>
</tr>
<tr>
<td>3.10. Materials</td>
<td>13</td>
</tr>
<tr>
<td>3.11. Methods</td>
<td>13</td>
</tr>
<tr>
<td>3.11.1. Sample preparation</td>
<td>13</td>
</tr>
<tr>
<td>3.11.2. Activated partial thromboplastin test APTT</td>
<td>14</td>
</tr>
<tr>
<td>3.11.3. Dilute Russell's Viper Venom Test (DRVVT)</td>
<td>15</td>
</tr>
</tbody>
</table>
# Chapter 4: Results and Discussion

## 4.1.1.1. Age of women with recurrent miscarriage  

## 4.1.1.2. Number of miscarriages  

## 4.1.1.3. Family history of miscarriage  

## 4.1.1.4. Age of fetal loss  

## 4.1.1.5. Pregnancies  

## 4.1.2.1. Frequency lupus anticoagulant (LA) in women with RM  

## 4.1.2.2.1. Correlation between LA in women with RM and their age  

## 4.1.2.2.2. Correlation between LA and family history of miscarriage  

## 4.1.2.2.3. Correlation between (LA) and pregnancies  

## 4.1.2.2.4. Correlation between (LA) age of fetal loss  

## 4.1.2.2.5. Correlation between LA and number of miscarriages  

## 4.2. Discussion  

## 5.1. Conclusions  

## 5.2. Recommendations  

References  

Appendix
**List of abbreviation**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM</td>
<td>Recurrent miscarriage</td>
</tr>
<tr>
<td>AT</td>
<td>Antithrombin</td>
</tr>
<tr>
<td>PC</td>
<td>Protein C</td>
</tr>
<tr>
<td>PS</td>
<td>Protein S</td>
</tr>
<tr>
<td>APC</td>
<td>Activated protein C</td>
</tr>
<tr>
<td>APS</td>
<td>Antiphospholipid syndrome</td>
</tr>
<tr>
<td>LA</td>
<td>Lupus anticoagulant</td>
</tr>
<tr>
<td>β 2GP1</td>
<td>Beta 2glycoprotein 1</td>
</tr>
<tr>
<td>ACL</td>
<td>Anti cardiolipin</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>PGL2</td>
<td>Prostacyclin</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TFI</td>
<td>Tissue factor inhibitor</td>
</tr>
<tr>
<td>TM</td>
<td>Thrombomodulin</td>
</tr>
<tr>
<td>TPA</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>VTE</td>
<td>Venous thromboembolism</td>
</tr>
<tr>
<td>LPD</td>
<td>Luteal phase defect</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovarian syndrome</td>
</tr>
<tr>
<td>HSV</td>
<td>Herpes simplex virus</td>
</tr>
<tr>
<td>FVL</td>
<td>Factor v Leiden</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematos</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ISTH</td>
<td>International Society on Thrombosis and Haemostasis</td>
</tr>
<tr>
<td>APTT</td>
<td>Activated partial thromboplastin Test</td>
</tr>
<tr>
<td>DRVVT</td>
<td>Dilute Russell's Viper Venom Test</td>
</tr>
</tbody>
</table>
## List of tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Frequency of lupus anticoagulant (LA) in women with RM</td>
<td>23</td>
</tr>
<tr>
<td>4.2</td>
<td>Relation between LA in women with RM and their age</td>
<td>23</td>
</tr>
<tr>
<td>4.3</td>
<td>Relation between LA and family history of miscarriage</td>
<td>24</td>
</tr>
<tr>
<td>4.4</td>
<td>Relation between (LA) and pregnancies</td>
<td>24</td>
</tr>
<tr>
<td>4.5</td>
<td>Relation between (LA) age of fetal loss</td>
<td>25</td>
</tr>
<tr>
<td>4.6</td>
<td>Relation between LA and number of miscarriages</td>
<td>25</td>
</tr>
</tbody>
</table>
**List of figure**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Age of women with recurrent miscarriage among study group</td>
<td>18</td>
</tr>
<tr>
<td>4.2</td>
<td>Number of miscarriages among study group</td>
<td>19</td>
</tr>
<tr>
<td>4.3</td>
<td>Family history of miscarriage among study group</td>
<td>20</td>
</tr>
<tr>
<td>4.4</td>
<td>Age of fetal loss among study group</td>
<td>21</td>
</tr>
<tr>
<td>4.5</td>
<td>Pregnancies among study group</td>
<td>22</td>
</tr>
</tbody>
</table>
CHAPTER ONE

INTRODUCTION

1.1 Overview:
Recurrence miscarriages (RM) defined as three or more consecutive pregnancy losses (Grandone et al., 2008). It affects about 5-15% of all pregnancies worldwide (Asherson RA et al., 2006). It has been considered as primary RM when pregnancy has never been carried to viability (Ahmed A et al., 2004) or secondary RM when a live birth has occurred at some time (Aznar J et al., 2005).

RM has been attributed to a large number of etiological factors, in approximately two-third of cases the cause is known to be genetic error, anatomic abnormalities of the reproductive tract, hormonal abnormalities, infection, immunologic factors, thrombophilia, systemic disease and idiopathic causes (Cosgriff TM et al., 2009). The term thrombophilia has been used to identify those disorders of haemostasis that are likely to predispose to thrombosis (BCSH 1990). More recently, it has been defined as a tendency to develop thrombosis as a consequence of predisposing factors that may be genetically determined, acquired, or both (Lane DA et al., 1996). The congenital and acquired conditions associated with venous thromboembolism include antithrombin (AT), protein C (PC), and protein S (PS) deficiencies and the activated PC (APC) resistance phenomenon, and among the acquired conditions associated with venous and arterial thromboembolism, a major role is played by the antiphospholipid antibody syndrome and moderate hyperhomocysteinemia (Haverkate F et al., 1995).

Antiphospholipid syndrome is an acquired condition, defined as the presence of thrombosis or pregnancy loss or maternal morbidity and persistent circulating antiphospholipid antibodies (APL) in plasma (O.A.Awodu et al., 2003).

Antiphospholipid antibodies (APA) comprises a heterogeneous group of autoantibodies directed against negatively charged phospholipids and include lupus anticoagulant (LA), Anti-β2-glycoprotein1 (β2GP1) and anticardiolipin antibodies (ACL) (Hanly JG, 2003).

Presence of lupus anticoagulant (LA) and anticardiolipin antibodies (ACA) increased incidence of RM (Aznar J et al., 2005). Also the presence of the antiphospholipid antibodies (APA) was shown to associate with recurrent miscarriage due to thrombosis of the uteroplacental vasculature and subsequent placental infarction (Kharnashta MA et al., 2009).
Several pathogenic mechanisms have been proposed, including inhibition of endothelial release of prostacyclin (Carreras LO et al., 1981), alterations in protein C-protein S pathway (Ruiz-Arguelles G et al., 1991), a direct procoagulant effect on platelets (Reverter JC et al., 1998), and impairment of fibrinolysis (Keeling DM et al., 1991).

Lupus anticoagulants (LA) which are Immunoglobulins usually IgG or, IgM or both directed against plasma proteins such as prothrombin or annexin V that are bound to phospholipids (D'Cruz DP et al., 2007).

The mechanism for the spontaneous recurrent miscarriage in patient with LA is still subject of research, but inhibition of prostacyclin production or release has since been postulated as likely pathogenic mechanism (O.A.Awodu et al., 2003). Researchers have found that antiphospholipid syndrome can increase women's chances of recurrent miscarriages. The reason for this is unclear; some researchers believe that antiphospholipid antibodies syndrome causes small blood clots to block the blood supply to the placenta. Others believe that having antiphospholipid syndrome may interfere with the fertilized egg’s ability to implant in the lining of the uterus. Antiphospholipid syndrome is well established as a cause of later miscarriages but doctors are still unsure of the role that antiphospholipid antibodies might play in early miscarriage (Rai. R.S, 2008).

1.2. Justification:

1.2.1. Recurrent miscarriage is a critical problem in which many factor play a crucial role such as antiphospholipid antibodies (lupus anticoagulant).

1.2.2. The determination of predisposing factor for recurrent miscarriages is useful to reduce the incidence of miscarriage in women.

1.2.3. The lupus anticoagulant (LA) has long been associated with increased risks of thrombosis and adverse obstetric outcomes including recurrent miscarriages.
1.3: Objectives:

1.3.1. General objective:
To Screen Lupus Anticoagulant in Women with Recurrent Miscarriage Attending Wad Medani Obstetrics and Gynecology Teaching Hospital Gezira.

1.3.2. Specific Objectives:

1.3.2.1. To determine the prevalence of APS in women with recurrent miscarriages using LA as a cause of unexplained recurrent miscarriage.

1.3.2.2. To determine the age, abortion number, family history of miscarriage, pregnancy, and age of fetal loss in women with recurrent miscarriage.

1.3.2.3. To detect the association between age, abortion number, family history of miscarriage, pregnancy, and age of fetal loss with lupus anticoagulant (LA) assay and positive cases.
CHAPTER TWO

LITERATURE REVIEW

2.1. Pregnancy:
Pregnancy refers to the fertilization and development of one or more offspring, known as a fetus or embryo, in a woman’s uterus. The term embryo is used to describe the developing offspring during the first 8 weeks following conception, and the term fetus is used from about 2 months of development until birth (MedicineNet.com, 2008). In a pregnancy, there can be multiple gestations, as in the case of twins or triplets. Childbirth usually occurs about 38 weeks after conception; in women who have a menstrual cycle length of four weeks, this is approximately 40 weeks from the last normal menstrual period. The World Health Organization (WHO) defines normal term for delivery as between 37 weeks and 42 weeks (WHO 2006).

Pregnancy is typically divided into three periods, or trimesters, each of about three months. In medicine, pregnancy is often defined as beginning when the developing embryo becomes implanted in the endometrial lining of a woman's uterus. The first 12 weeks of pregnancy are considered to make up the first trimester. According to the American Pregnancy Association, by the end of the first trimester, the fetus will be about 3 inches (76 mm) long and will weigh approximately 1 ounce (28 gm.) (American Pregnancy Association 2010). Weeks 13 to 28 of the pregnancy are called the second trimester. By the end of the second trimester, the expanding uterus has created a visible "baby bump". The third trimester of pregnancy spans from week 28 to the birth. The woman's belly will transform in shape as the belly drops due to the fetus turning in a downward position ready for birth (MedicineNet.com, 2008).

2.2 Normal haemostatis:
The normal haemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors (Hoffbrand AV et al., 2006).

2.2.1. Blood vessels:
An immediate vasoconstriction of the injured vessel and reflex constriction of adjacent small arteries and arterioles is responsible for an initial slowing of blood flow to the area of injury. When there is widespread damage this vascular reaction prevents exsanguination. The reduced blood flow allows contact activation of platelets and coagulation factors. The vasoactive amines
and thromboxane A2 liberated from platelets, and the fibrinopeptides liberated during fibrin formation, also have vasoconstrictive activity.

The endothelial cell in blood vessels has an active role in the maintenance of vascular integrity. 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 PGl2 and NO, which have vasodilatatory properties and inhibit platelet aggregation (Hoffbrand AV et al., 2006).

2.2.2 Platelets:
The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. The immobilization of platelets at the sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet-platelet (aggregation) interactions. The blood flow conditions determine the specific receptor ligand interactions (Hoffbrand AV et al., 2006).

2.2.3. Coagulation Cascade:
Tissue factor (TF), previously known as factor III of the extrinsic pathway is the primary initiator of coagulation in both normal and disease states. Tissue factor is the only membrane bound member of the coagulation cascade; it is an extremely pro-coagulant molecule that also has cell signaling actions and interacts with numerous pathways other than coagulation.

When TF comes into contact with blood it binds and activates FVII in the presence of calcium, this TF-VIIa-Ca2+ complex then activates factors IX and X the initiation phase. Factor Xa leads to the generation of thrombin but tissue factor pathway inhibitor (TFPI) rapidly inactivates this part of the pathway such that only traces of thrombin can be produced. Activated FIX in addition to FVa and FVIIIa generated by the trace levels of thrombin allow amplification of the pathway. This ‘propagation’ of thrombin formation by the intrinsic pathway is an essential component of the secondary coagulation response in order to achieve effective hemostasis (Hoffbrand AV et al., 2006). In the normal state TF is constitutively expressed in the extravascular tissues. There are high levels in the adventitia of blood vessels, fibrous capsules of organs and the epithelium of the skin and internal mucosal layers. This constitutive TF is found in close proximity to the vascular space where it is responsible for appropriate activation of coagulation in response to an interruption to vascular integrity. There are several cells which can be induced to express TF including endothelial cells, smooth muscle cells and circulating monocytes. Endotoxin, tumor
necrosis factor alpha (TNF-a), lipoproteins and growth factors can all stimulate intravascular TF expression. Induced TF is present in the intravascular space where it can initiate pathologic coagulation. It is this induced TF expression that is believed to play a key role in numerous disease states including sepsis and atherosclerosis. At the culmination of the coagulation cascade, thrombin cleaves fibrinogen into fibrin monomers which polymerize spontaneously but need to be cross linked to produce a stable clot. Factor XIII (plasma transglutaminase) is also activated by thrombin and stimulates the formation of covalent bonds between adjacent fibrin molecules completing the process of secondary coagulation (Hoffbrand AV et al., 2011).

2.2.4 Coagulation inhibitors:

2.2.4.1. Tissue Factor Inhibitor:
Tissue factor inhibitor (TFI) is a serine protease inhibitor produced primarily by endothelial cells of the microvasculature. It directly inhibits factor Xa and the factor VIIa – TF complex. It is the only endogenous inhibitor of TF-VIIa making it an extremely important component of normal hemostatic balance (Hoffbrand AV et al., 2011).

2.2.4.2. Antithrombin:
Antithrombin (AT) is a broad spectrum serine protease inhibitor found in plasma and is produced by the liver. It has both anti-coagulant and anti-inflammatory effects. Antithrombin inhibits the action of thrombin, VIIa, IXa, Xa, XIa and XIIa. The AT molecule binds the coagulation factor in a 1:1 ratio leading to factor inactivation, the complex is subsequently removed by the reticuloendothelial system (Hoffbrand AV et al., 2011).

2.2.4.3. Protein C System:
Protein C and its cofactor protein S are vitamin K dependent serine proteases. Protein C has potent anticoagulant, profibrinolytic and anti-inflammatory actions. Protein C is a plasma protein produced by the liver which circulates in an inactive form. Thrombomodulin (TM), an endothelial membrane bound protein complexes with and inactivates thrombin. The TM-thrombin complex can then rapidly binds and activate protein C. Another endothelial membrane bound protein the endothelial protein C receptor (EPCR) potentiates this process by concentrating protein C around TM. Once activated, protein C is then released back into circulation where it has anti-coagulant effects. The binding of thrombin to TM effectively converts thrombin from a potent pro-thrombotic factor to a potent anti-thrombotic factor.
Activated protein C in association with its cofactor protein S inactivates factors Va and VIIIa (Hoffbrand AV et al., 2011).

2.2.4. Fibrinolytic pathway:
Fibrinolysis is often forgotten when the normal coagulation cascade is considered but removal of fibrin is vital to maintaining hemostasis without producing thrombosis. Plasmin is responsible for the degradation of fibrin. Plasmin is cleaved from plasminogen (which is bound to fibrin within the clot) by tissue plasminogen activator (TPA) and/or urokinase. These are produced and released by endothelial cells in response to injury or thrombin. In addition to fibrin, plasmin also degrades FVa and FVIIIa (Hoffbrand AV et al., 2011).

2.3. Pregnancy and haemostasis:
Pregnancy is a hypercoagulable state, which may impair placental flow and then its function and fetal growth and may predispose to develop venous thrombosis. During pregnancy, in fact, many changes have been observed in the haemostatic balance with a trend towards thrombophilia in order to be prompt for the haemostatic challenge of delivery (Abbate R et al., 2003). Pregnancy is a condition associated to thrombophilia, and for this reason it is associated with the increase of several clotting factors (i.e. factor VIII, VWF, fibrinogen, factor VII) (Hathaway WE and Goodnight SH Jr, 1993). Moreover, other markers of a hypercoagulable state are also increased during pregnancy, such as D-dimer and/or prothrombin fragment (Boer K et al., 1989). For this reason, episodes of venous thromboembolism (VTE) have been observed during pregnancy (Colman-Brochu S et al., 2004). Moreover, women carrying further thrombotic risk factors (e.g. acquired thrombophilia) show an additionally increased risk of thrombotic events during pregnancy, such as venous thromboembolism and/or miscarriage (Robertson L et al., 2006).

2.4. Recurrent miscarriages:
Abortion is termination of pregnancy before 20 weeks (some say 24 weeks) gestation and/or fetus/embryo weighing ≤500g. Recurrent miscarriages (RM) defined as three or more consecutive pregnancy losses (Asherson RA et al., 2006). It affects about 5-15% of all pregnancies worldwide (Ahmed A et al. 2004). The purported causes of recurrent miscarriage are multiple, ranging from genetic, environmental, infectious, metabolic, and endocrine to purely anatomic ones. The best defined causes are parental chromosomal abnormalities, metabolic abnormalities, and anatomic abnormalities.
2.4.1 Genetic etiologies:
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 mosaics. Single gene defects, such as those associated with cystic fibrosis or sickle cell anemia, are seldom associated with RM (Stephenson MD et al., 1996).

2.4.2 Anatomic etiologies:
Anatomic abnormalities account for 10% to 15% of cases of RM. These include congenital uterine anomalies, intrauterine adhesions, and uterine fibroids or polyps (Lin PC; 2004). Other Müllerian anomalies, including unicornuate, didelphic, and bicornuate uteri have been associated with smaller increases in the risk for RM (Raga F et al., 1997).

2.4.3 Endocrine etiologies:
Luteal phase defect (LPD), polycystic ovarian syndrome (PCOS), diabetes mellitus, thyroid disease, and hyperprolactinemia are among the endocrinologic disorders implicated in approximately 17% to 20% of RM (Fox-Lee L and Schust DJ; 2007). Also, untreated hypothyroidism is clearly associated with spontaneous miscarriage (Vaquero E et al., 2000).

2.4.4 Environmental etiologies:
Environmental exposures to organic solvents, medications, ionizing radiation, toxins smoking, alcohol, and caffeine have been associated with higher rates of spontaneous miscarriage (Washington, DC: 2001).

2.4.5 Infectious etiologies:
The role of infectious agents in recurrent miscarriage is less clear, with a proposed incidence of 0.5% (Stephenson MD et al., 1996) to 5% (Fox-Lee L and Schust DJ; 2007). Certain infections, including Listeria monocytogenes, Toxoplasma gondii, rubella, herpes simplex virus (HSV), measles, cytomegalovirus, are known or suspected to play a role in RM (Abel E. L et al., 1997).

2.4.6 Immunologic etiologies:
Because a fetus is not genetically identical to its mother, it is reasonable to infer that there are immunologic events that must occur to allow the mother to carry the fetus throughout gestation without rejection. In fact, there have been at least 10 such mechanisms proposed (Robertson L et al., 2006). It therefore follows that there may be abnormalities within these immunologic
mechanisms that could lead to both sporadic and recurrent pregnancy loss. Despite the intense interest in this potential etiology for RM, there is no consensus on appropriate diagnostic workup or therapy. Therapies such as paternal leukocyte immunization, intravenous immune globulin, third-party donor cell immunization, and trophoblast membrane infusions have been shown to provide no significant improvement in live birth rates (Thellin O et al., 2000). One specific autoimmune disorder, APS, it has been clearly linked with many poor obstetric outcomes, including RM (Porter TF et al., 2006).

2.4.7. Thrombotic etiologies:

Thrombophilia refer to a group of prothrombotic conditions in which there is an increased propensity to clot formation at various site in human circulation. The consequence of such disorders is enhanced prothrombotic tendency affecting the uteroplacental circulation and they have been implicated as a possible cause of recurrent miscarriage and pregnancy loss. Thrombophilia has been identified as one of the main causes of RM until 40%, in particular early RM (Brenner B et al., 1999). It may inherited thrombophilia, acquired thrombophilia and combined thrombophilia (Franchini M and Veneri D; 2005).

2.4.7.1. Inherited thrombophilia:

Thrombophilia are genetic conditions that increase risk of thromboembolic disease. Inherited thrombophilia may be due to deficiency of clotting inhibitors or to gene variants leading to a hypercoagulable tendency. Gene variants frequently associated with RM are prothrombin A20210G and/or factor V Leiden. Prothrombin A20210G has been identified as a risk factor for pregnancy loss in several studies and has been associated mainly to early RM (Pickering W et al., 2001). On the other hand, factor V Leiden, which is responsible for more than 75% of inherited activated protein C resistance, is the more commonly inherited thrombotic risk factor associated to RM (Rai R et al., 2001).

In particular, a case control study by Ridker et al. has reported an increased prevalence of FVL in women with RM, while other studies revealed a strong relationship between FVL and early RM (Ridker PM et al., 1998). FVL has been identified as a risk factor also for late RM. Also, deficiency of clotting inhibitors, such as protein S, protein C and/or antithrombin, has been clearly associated to RM since 1996 (Pabinger I et al., 2000).
2.4.7.2. Acquired thrombophilia:
The most common Acquired thrombophilia include acquired hyperhomocystinaemia, activated protein C resistance and antiantiphospholipid syndrome.

2.5. Antiantiphospholipid syndrome:
The antiphospholipid antibody syndrome is an acquired disorder characterized by multiple antibodies that are associated with both arterial and venous thrombosis and infarction in the placenta. Antiphospholipid antibodies are a family of approximately twenty autoantibodies directed against phospholipid binding plasma protein. There are two most clinically important antiphospholipid antibodies include, Lupus anticoagulant (LA) and Anticardiolipin antibodies (ACA). Three Clinical features (venous or arterial thrombosis, recurrent fetal loss and thrombocytopenia) in conjunction with positive laboratory findings (positive IgG or IgM anticardiolipin antibodies (ACA) or positive lupus anticoagulants (LA), will satisfy the criteria for the diagnosis of antiphospholipid antibody syndrome. These women have only a 10% live-birth rate in subsequent pregnancies in which no pharmacological treatment is given (Sanson BJ et al., 1996).

2.5.1. Lupus anticoagulant (LA):
Lupus anticoagulant LA are antibodies which are not directed at any one of the coagulation factors but still interfere with coagulation in vitro, they are usually not associated with bleeding (Feinstein DI and Rapaport SI; 1972). In 1952 lupus anticoagulant was first described by Conley and Hartman in two patients with systemic lupus erythematosus and it was erroneously named lupus anticoagulant (Johansson E and Lassus A; 1974). In 1972 the term lupus anticoagulant (LA) was proposed by Fainstein and Rapaport (Laurell AB and Nilsson IM; 1957). Early cases reports of LA emphasized the frequent association with biological false positive tests for syphilis, a reaction that depend on the presence of cardiolipin (Thiagarajan P et al., 1980), Laurell and Nilsson then found that anticardiolipin could adsorb LA from plasma (Triplelt DA et al., 1988), subsequently Thiagrajan et al. studied patient with Waldenstrom' s macroglobulinemia and monoclonal IgM with LA activity (Nilsson IM et al., 1975). Lupus anticoagulant may be association with various clinical complications includes arterial and venous and spontaneous miscarriage, the accumulative literature suggests that approximately 30% of paients with LA have least one thrombotic event (Firkin BG et al., 1980). Nilson et al. were the
first to report an association between LA and intrauterine death (Nilsson IM et al., 1975). This observation has since been confirmed by a number of other investigations (Carreras LO and Vermyleen JG; 1982).

Fetal growth retardation, first trimester abortion and unexplained death in the second and third trimester are more frequently seen in women with LA and/or Anticardiolipin antibodies (ACA). In many cases the placenta has been found to have extensive infraction. Frequency of Lain women with history of spontaneous abortion or intrauterine death was found to be 11/63 (17%) in a prospective study by (Moroz LA et al., 1986).

2.5.1.1. Pathophysiology of LA:

- Now it is clear that LA react with phospholipid, a number of studies have focused on pathogenesis of thrombosis in patients with LA. Since phospholipids are present in all cell membranes, potential membrane damage to endothelial cells or platelets could explain thromboembolic complications associated with LA. Carreras et al. were first to propose a possible effect of LA on endothelial cell membranes (Carreras LO et al., 1981).

- Subsequent studies confirmed the ability of LA to inhibit production of prostacycllin (PGL2) in vitro (Carreras LO and Vermyleen JG; 1982), which is synthesized by vascular endothelial cells. It is a potent inhibitor of platelet aggregation and prevents their deposition on normal vascular endothelium.

- LA can also reduce fibrinolytic activity Angles- Canho et al. found reduced fibrinolytic activity in 12 of 28 patients with LA (Moroz LA et al., 1986) and alterations in protein C-protein S pathway.

- Inhibition of Annexin V5, which appears to play a thrombomodulatory role in the placental circulation where it is necessary for maintenance of placental integrity (Satoh A et al., 1999). Patients with LA have specifically recognized annexin A5 and increased thrombosis (Nojima J et al., 2001).

- Inhibition of β2GPI anticoagulant activity, β2GPI is a highly glycosylated single-chain-protein present in plasma that avidly binds to negatively charged phospholipids such as cardiolipin, phosphatidylserine or phosphatidylinositol (Wurm H; 1984.). The physiological function of β2GPI is uncertain. This protein exhibits anticoagulant properties; it inhibits platelet aggregation induced by ADP (Nimpf J et al., 1985),
intrinsic coagulation pathways (Schousboe I; 1985), the prothrombinase activity of platelets (Nimpf J et al., 1986), and the activation of protein C in the presence of phospholipids (Keeling DM et al., 1993). However, familial deficiency of β2GPI is not a risk factor for thrombosis (Bancsi LF et al., 1992).

- Inhibition of Antithrombin Activity, Antithrombin is the major inhibitor of factors IXa, Xa, and thrombin. Heparan sulphate proteoglycan is expressed on vascular endothelium and plays an important role in vascular structure and function. Vascular heparan sulphate proteoglycan is required for the activation and optimal anticoagulation activity of antithrombin. Autoantibodies to heparan sulphate proteoglycan have been detected in plasma of patients with SLE (Fillit H et al., 1993). An LA patient with normal antigenic levels of antithrombin, but low functional activity, has also been reported (Cosgriff PM and Martin BA. 1981).
CHAPTER THREE
MATERIALS AND METHODOLOGY

3.1. Study design:
This was a descriptive cross sectional hospital based study.

3.2. Study citing:
The study was conducted in Wad Medani, Gezira state, Sudan. It is a capital city of the state, this town lies on the western bank of the Blue Nile River, at a junction of highways. This descriptive study was conducted in Wad Medani Obstetrics and Gynecology Teaching Hospital; it is a tertiary referral hospital delivering approximately 4,000 patients per annum. The hospital admitted different patients coming from all over Gezira state and other neighboring state.

3.3. Study population:
This study was conducted at Wad Medani Obstetrics and Gynecology Teaching on women with history of spontaneous recurrent miscarriage who attended the hospital.

3.4. Study period:
The study was conducted during the period between (January - April 2015).

3.5. Inclusion criteria:
Patients with three or more recurrent miscarriage, with or without previous living babies.

3.6. Exclusion criteria:
Patients with history of three or more miscarriages post 12 weeks of miscarriage were excluded from this study due to a coagulation disturbance at this period. Medical termination, e.g. for molar pregnancy or for grossly abnormal babies or due to maternal disease. All other reasons for RM were excluded e.g. renal disease, Genetic disorder, uterine abnormalities, Diabetes mellitus, Toxoplasma...etc.

3.7. Sampling and sample size:
3 ml of venous blood was collected from 50 women who had history of spontaneous recurrent miscarriages and the blood sample were added to a tube containing trisodium citrate (3.2%).
3.8. Ethical consideration:

The ethical clearance of this study was taken from Ministry of Health Authorities, Gezira State, Permission from the head director of Wad Medani Obstetrics and Gynecology Teaching Hospital and written consent from women included in the study.

3.9. Data collection:

3.9.1. Questionnaire interview:

A meeting interview was used for filling in a questionnaire which designated for matching the study need. All interviews were conducted face to face by the researcher herself.

3.9.2. Specimen collection:

Blood samples were collected from 50 women with history of 3 or more recurrent miscarriages after 12 weeks from miscarriage, 3 ml of venous blood were drawn in trisodium citrate (3.2%). Centrifuged immediately after collection at ≤ 1500 g for 15 minutes to obtain Platelet Poor Plasma and stored in capped tubes at 40°C.

3.10. Materials:

Cotton, alcohol, syringes, centrifuge, Yellow tips, trisodium citrate tubes, pepite, coagulation analyzer, caps, APTT reagent, LA screen and LA confirm reagents.

3.11. Methods:

3.11.1. Sample preparation:

3ml of freshly collected patient's blood was mixed in tri sodium citrate (3.2%) tube. Centrifuged immediately after collection at ≤ 1500 g for 15 minutes to obtain Platelet Poor Plasma. Store in capped tubes at 40°C, the samples were frozen, so the plasma was centrifuged again to remove platelets. Screening for LA performed by the Activated Partial Thromboplastin Time (APTT) and by the Dilute Russell’ s Viper Venom Time (DRVVT) according to recommendations of International Society on Thrombosis and Haemostasis (ISTH), automated coagulometer was used.

3.11.2. Activated Partial Thromboplastin Time (APTT):

3.11.2.1. Principle of APTT:

The test measures the clotting time of plasma after the activation of contact factors and the addition of phospholipid and CaCl2, but without added tissue thromboplastin, and so indicates
the overall efficiency of the intrinsic pathway. To standardize the activation of contact factors, the plasma is first preincubated for a set period with a contact activator such as kaolin, silica or ellagic acid. During this phase of the test, factor XIIa is produced, which cleaves factor XI to factor Xla, but coagulation does not proceed beyond this in the absence of calcium. After recalcification, factor Xla activates factor IX and coagulation follows. A standardized phospholipid is provided to allow the test to be performed on PPP. The test depends not only on the contact factors and on factors VIII and IX but also on the reactions with factors X, V, prothrombin and fibrinogen. It is also sensitive to the presence of circulating anticoagulants (inhibitors) and heparin.

3.11.2.2. Test procedure:
50μl of patient citrated platelet poor plasma were added into a warmed cuvette, and then 50μl of thromboplastin reagent was added, mixed and incubated for 2−3 minutes. Following that, 50μl of CaCl2 was added and the end point was observed. The mean of the double determination was plotted. Reference value for APTT is 30 to 40 seconds.

3.11.3. Dilute Russell's Viper Venom Test (DRVVT):

3.11.3.1. Principle DRVVT:
Russell's viper venom directly activates factor X in the presence of phospholipid and calcium ions, by passing factor VII of the extrinsic pathway and the contact and antihaemophilic factors of the intrinsic pathway. In normal plasma in the absence of lupus 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 there by preventing the activation of prothrombinase complex. This results in a prolongation of clotting time with LA reagent.

3.11.3.2. Reagent preparation:
- All reagents were put at room temperature (about 25°C) prior to reconstitution.
- 1 ml of distilled water was added to the R1 (LA screen) and R2 (LA confirm) reagent.
- Gently mixed to dissolve and Kept for 15−20 minutes at room temperature. Mixed again gently ensuring complete re-suspension of the lyophilized material.
- Mixed well before withdrawing material every time for testing.
3.11.3.3. Test procedure:
All reagents were put at room temperature (about 25°C) before pre warming at 37°C for testing. Calcium Chloride was incubated at 37°C for 10 minutes. 50μl of patient citrated platelet poor plasma were added into a warmed cuvette, then 50μl of R1 (LA screen) reagent was added and mixture was incubated for 1–2 minutes. Following that, 50μl of cacl2 was added and the end point was observed, this is the screen time for the plasma specimen. If screen time is less than 45 seconds, it was indicated absence of LA and there is no needed to performed confirmatory test. When the screen time is more than 45 seconds the test procedure was repeated for the sample used R2 (LA confirm) reagent.

3.11.2.4. Interpretation of results:
The normal expected value for screen time is 28-45 seconds. The normal expected value for confirm time is 28-40 seconds. FORTRESS’s LA kit is based upon the ratio of clotting time using LA screen reagent and clotting time of the same sample using LA confirm reagent.

Mean Screen Time

\[
\text{Ration (R)} = \frac{\text{Mean Screen Time}}{\text{Mean Confirm Time}}
\]

<table>
<thead>
<tr>
<th>Ratio (R)</th>
<th>R &lt; 1.3</th>
<th>R = 1.5-1.8</th>
<th>R = 1.8-2.4</th>
<th>R&gt;2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpretation of results</td>
<td>Normal LA</td>
<td>Moderate LA</td>
<td>High LA</td>
<td>High LA</td>
</tr>
</tbody>
</table>

If results were borderline, (ratio of 1.3-1.4) mixing studies were done further with the sample specimen. This test was carried out on a 50:50 mixture of test plasma and normal plasma.

3.12. Statistical analysis:
Data collected was analyzed by using the Statistical Package for the Social Science software (SPSS version 20).
CHAPTER FOUR

RESULTS AND DISCUSSION

The result of the analysis of the 50 participants (pregnant and post miscarriage) with history are the following:

4.1. Age of women with recurrent miscarriages:

The age of the study participants was between 20 and 50 years, out of 50 participants, 33 (66%) of women with recurrent miscarriage their age between (31-40) years, (Figure 4.1)

![Figure 4.1 Age of women with recurrent miscarriage](image)

4.2. Number of miscarriages among study group:

The number of miscarriage between the study participants ranging from 3 to 11; they were divided in the following groups: 3 miscarriages include 34 (68%) participants, 4 miscarriages include 8 (16%) participants, from 5 – 8 miscarriages include 6 participants and from 10 – 11 miscarriages include 2 participants (Figure 4.2).
Figure (4.2) Number of miscarriages among study group

4.3. Family history of recurrent miscarriages among study group:

Out of 50 participants 14 (28%) had positive family history of recurrent miscarriage (Figure 4.3)

4.3. Family history of recurrent miscarriage
4.4. Age of fetal loss among study group:

Out of 50 participants 22 (44%) had history of fetal loss in the second trimester (14 – 26) weeks, 20 (40%) had history of fetal loss in the first trimester (1 – 13) weeks and 8/50 (16%) had history of fetal loss in the third trimester (27 – 39) weeks (Figure 4.4)

![Age of fetal loss among study group](image)

**Figure (4.4) Age of fetal loss among study group**

4.5. Pregnancy among study group:

The study include 46/50 (92%) post miscarriage and 4/50 (8%) were pregnant, (Figure 4.5)

![Pregnancy among study group](image)

**Figure (4.5) Post miscarriage and pregnant ladies distribution**
Table (4.1) Frequency of lupus anticoagulant (LA) in women with RM:

<table>
<thead>
<tr>
<th>Result of LA</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3</td>
<td>6.0%</td>
</tr>
<tr>
<td>Negative</td>
<td>47</td>
<td>94.0%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table (4.1) shows that 3/50 (6%) of women with recurrent miscarriage in Wad Medani Teaching Hospital of Obstetrics and gynecology were positive LA and 47/50 (94%) were negative. From the same table the prevalence of APA using LA as cause of recurrent miscarriage in Wad Medani Teaching Hospital of Obstetrics and Gynecology was 6%. This study was found the frequencies Lupus Anticoagulant in women with recurrent miscarriage were 3/50 (6%) which agreement with studies were detected LA 5/50 (10%), (Das I et al. 1991), (4.35%) of LA among Nigerians women with history of recurrent miscarriage (Owodu OA et al., 2003), LA 27/200 (13.5%) in Iraqi women (Nasir AS Al-Allawi et al. 2012). LA was detected in 5 of 50 cases 10% (Das I et al. 1991) and study was done in Wad Medani Obstetric and Gynecological Hospital, Sudan, LA was found 2/50 (4%) in women with recurrent miscarriage (Abdelnassir MA et al., 2014).

The study in disagreement with study conducted in an Indian 235 women with recurrent miscarriage, frequencies of LA were 25% (Velayuthaprabhu S et al., 2005). In another study from Iran included 138 women with RM, the frequency of LA was 18.6% (Zolghadri et al 2004), (Vaidyanathan et al. 2011) from Oman found the frequency and LA was (18.2%). the disagreement may be due to the difference in sample size, environmental, and genetic difference. Also the study in disagreement with study was done in 100 women with recurrent miscarriage the presence of lupus anticoagulant (LA) was 20/100 (20%), (Jevara MK et al., 2013); the disagreement may be due to the difference in sample size.
Table (4.2) Relation between LA in women with recurrent miscarriage and their age:

<table>
<thead>
<tr>
<th>Age</th>
<th>Result of (LA)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>(20- 30) years</td>
<td>1(33.3%)</td>
<td>11(23.4%)</td>
</tr>
<tr>
<td>(31- 40 ) years</td>
<td>2(66.7%)</td>
<td>31(66%)</td>
</tr>
<tr>
<td>( 41- 50 ) years</td>
<td>0(0%)</td>
<td>5(10.6%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3(100%)</strong></td>
<td><strong>47(100%)</strong></td>
</tr>
</tbody>
</table>

Table (4.2) shows that (33.3%) of women with recurrent miscarriage in the age between (20- 30) years were positive for lupus anticoagulant (LA) and (66.7%) of positive cases their age between (31- 40) years. The study reported there was no statistical significant association between positivity of lupus anticoagulant (LA) in women with recurrent miscarriage and their age Chi-Square = 0.435 and p value = 0.8), and this may be due to all participants with in the child birth age.

Table (4.3) Relation between LA and family history of miscarriage:

<table>
<thead>
<tr>
<th>Family history</th>
<th>Result of (LA)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Yes</td>
<td>3(100%)</td>
<td>11(23.4%)</td>
</tr>
<tr>
<td>No</td>
<td>0(0%)</td>
<td>36(76%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3(100%)</strong></td>
<td><strong>47(100%)</strong></td>
</tr>
</tbody>
</table>

Table (4.3) shows that 3/50 (100%) of women with recurrent miscarriage whom they have family history of miscarriage were positive for lupus anticoagulant( LA) In this study 14/50, (28%) of positive patients have family history of recurrent miscarriage and there was a significant association between family histoty of RM and positivity of LA (Chi – square = 8.207 and p value=0.04), it is similar to study was conducted in Wad Medani Obstetric and Gynecology Teaching Hospital, Sudan, by Abdelnassir which found that 10/50, (20%) of the positive cases of LA had family history of RM (Abdelnassir MA et al., 2014). This may due to genetic predisposition to the development of APA (LA), approximately two-third of recurrent
miscarriage cases the cause is known to be genetic error (Cosgriff TM et al., 2009).

**Table (4.4) Relation between (LA) and pregnancy:**

<table>
<thead>
<tr>
<th>Pregnant.</th>
<th>Result of (LA)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Pregnant</td>
<td>1(33.3%)</td>
<td>3(6.4%)</td>
</tr>
<tr>
<td>Non pregnant</td>
<td>2(66.7%)</td>
<td>44(93.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>3(100%)</td>
<td>47(100%)</td>
</tr>
</tbody>
</table>

Table (4.4) shows that 1/50 (33.3%) of women with recurrent miscarriage whom they pregnant were positive for lupus anticoagulant (LA), and 2/50 (66.7%) of positive cases were non-pregnant. This result is similar to study was done by (Abdelnassir MA et al. 2014) which was found no statistically significant association between positivity of LA and Pregnancy (Chi – square= 2.783 and p value = 0.9). This is most probably due to maternal antiphospholipid antibodies down regulation during pregnancy.

**Table (4.5) Relation between (LA) and ages of fetal loss:**

<table>
<thead>
<tr>
<th>Age of fetal loss.</th>
<th>Result of (LA)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>(1-13 ) weeks</td>
<td>3(100%)</td>
<td>17(36.2%)</td>
</tr>
<tr>
<td>(14-26 ) weeks</td>
<td>0(0%)</td>
<td>22(46.8%)</td>
</tr>
<tr>
<td>(27-39 ) weeks</td>
<td>0(0%)</td>
<td>8(17.%)</td>
</tr>
<tr>
<td>Total</td>
<td>3(100%)</td>
<td>47(100%)</td>
</tr>
</tbody>
</table>

Table (4.5) shows that 3/50 (100%) of women with recurrent miscarriage whom their age of fetal loss in (1-13) weeks were positive for lupus anticoagulant (LA). The study was found there was a significant relationship between positivity of lupus anticoagulant (LA) in women with recurrent miscarriage and age fetal loss (Chi – square =9.787 and p value=0.03).
Table (4.6) Relation between LA and number of miscarriages:

<table>
<thead>
<tr>
<th>Number of abortion</th>
<th>Result of (LA)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Three</td>
<td>2 (66.7%)</td>
<td>32 (68.1%)</td>
</tr>
<tr>
<td>Four</td>
<td>0 (0%)</td>
<td>8 (17%)</td>
</tr>
<tr>
<td>Five</td>
<td>0 (0%)</td>
<td>2 (4.3%)</td>
</tr>
<tr>
<td>Six</td>
<td>1 (33.3%)</td>
<td>1 (2.1%)</td>
</tr>
<tr>
<td>Eight</td>
<td>0 (0%)</td>
<td>2 (4.3%)</td>
</tr>
<tr>
<td>Ten</td>
<td>0 (0%)</td>
<td>1 (2.1%)</td>
</tr>
<tr>
<td>Eleven</td>
<td>0 (0%)</td>
<td>1 (2.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (100%)</td>
<td>47 (100%)</td>
</tr>
</tbody>
</table>

Table 4.6 shows that (66.7%) of positive LA had three miscarriages and (33.3%) of positive LA had six miscarriages. The study was reported there was no statistically significant association between positivity of LA and number of miscarriages (Chi – square = 5.760 and p value=0.25).
CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. Conclusions:

- The occurrence of lupus anticoagulant (LA) was (6%) among women with recurrent unexplained miscarriage in Wad Medani Teaching Hospital of Obstetrics and Gynecology, the prevalence of APS was (6%) by using LA as a cause of unexplained recurrent miscarriage.
- There was a significant association between family history of recurrent miscarriage and positivity of LA \( (p \text{ value} = 0.04) \).
- There was a significant relationship between the presence of lupus anticoagulant (LA) in women with RM and age fetal loss \( (p \text{ value} = 0.03) \).
- There was no association between age of women, pregnancy, number of miscarriage and positivity of LA.

5.2. Recommendations:

- Screening for lupus anticoagulant (LA) should be part of routine investigation for women with unexplained recurrent miscarriage.

- Further studies with large samples size are highly recommended to support the finding of this study.
REFERENCES:


Appendix
Questionnaire of Lupus Anticoagulant among women with recurrent miscarriage

S.No:…………………………

Personal data
Name:…………………………………………………………………………………………………….
Age:………………………………………………Residence:………………………………………..
Tel………………………………………………………………………………………………………

Medical history of:
Diabetic Yes………. No……….  
Hypertension Yes………. No……….  
Joint pain Yes………. No……….  

Pregnancy morbidity:
The number of miscarriage…………………times.
The time of last abortion before……………….
Age of fetal loss………………..

Family history of:
Recurrent miscarriage Yes………. No……….  
Venous thromboembolic disease Yes………. No……….  
Arterial thromboembolic disease Yes………. No……….  

Drug history of:
anticoagulant therapy Yes………. No………. Contraceptive therapy Yes………. No……….  
Hormones therapy Yes………. No……….  

Result:
PTT: Normal…………………………Prolonged…………………………

LA result:
High………………… Moderate………………… Low……………………