

Research Article**Antiphospholipid Syndrome among Women with Unexplained Recurrent Abortion in Wad Medani Obstetrics and Gynecological Teaching Hospital, Gezira State, Sudan****Abdelnassir M. Ahamed^{1*}, Awad Elseed Mustafa Elsaheed², Mohamed Elsanousi Mohamed³, Ahmed A. Mohamedani², GadAllah Modawe⁴**¹University of west Kurdofan, Faculty of Medicine and Health sciences, Department of Pathology, Elnuhoud, Sudan²University of Gezira, Faculty of Medicine, Department of Pathology, WadMedani, Sudan³University of Gezira, Faculty of Medicine, Department of Obstetrics and Gynecology, Wad Medani, Sudan⁴Omdurman Islamic University, Faculty of Medicine and Health sciences, Department of Biochemistry, Omdurman, Sudan***Corresponding author**

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Abstract: Recurrent miscarriage affects 2–5% of the population. The causes of recurrent miscarriage are multiple ranging from genetic, environmental, infectious, metabolic, and endocrine to purely anatomic ones. Pregnancy leads to a hypercoagulable state with consequent increased risks of thromboembolism and disseminated intravascular coagulation. Antiphospholipid (aPL) syndrome (APS) represents a significant cause of recurrent miscarriage. This study aimed to determine the prevalence of antiphospholipid syndrome, in 50 (5 pregnant and 45 post miscarriage) Sudanese women, with recurrent unexplained miscarriages. Using lupus-anticoagulant test and anticardiolipin antibody ELISA assay. Questionnaires were filled, from the participants and each one tested for lupus anticoagulant, complete blood count (CBC) and anticardiolipin antibodies. Thirteen (26%) of the 50 participants were positive for aPL antibodies. Statistically significant associations were observed between aPL antibodies and the use of oral contraceptive pills and between aPL antibodies and the participants tested in post miscarriage period.**Keywords:** Recurrent miscarriage, Antiphospholipid, Lupus-Anticoagulant, Anticardiolipin

INTRODUCTION

Abortion is defined as termination of pregnancy resulting in expulsion of an immature, nonviable fetus. A fetus of <20 weeks gestation or a fetus weighing <500 gm is considered an abortus [1]. Miscarriage is the commonest complication of pregnancy. Recurrent miscarriage is usually defined as the loss of three or more consecutive pregnancies [2], the difference between sporadic and recurrent miscarriage is important. It helps us to predict the chance of a successful pregnancy in the future, and the likelihood of there being a recurring cause for the loss of the pregnancy. A woman who has suffered a single sporadic miscarriage has 80% chance and a woman with three consecutive miscarriages 60% chance of her next pregnancy being successful [2]. The causes of recurrent abortion are ranging from genetic factors, hereditary thrombophilia, endocrine and metabolic disorders, uterine abnormalities [3], and others. The term thrombophilia has been used to identify those disorders of haemostasis that are likely to predispose to thrombosis [4]. 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 [5]. 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 [6]. Antiphospholipid (aPL) syndrome (APS) is an acquired thrombophilic disorder in which patients have vascular thrombosis and/or pregnancy complications attributable to placental insufficiency, accompanied by laboratory evidence for the presence of antiphospholipid antibodies in blood. The disorder is referred to as primary APS when it occurs in the absence of systemic lupus erythematosus (SLE) rheumatoid arthritis HIV and hepatitis C virus, or secondary APS in their presence [7]. Antiphospholipid antibodies are a family of antibodies reactive with proteins that have the property of binding to negatively

charged phospholipids. The most important is B2 glycoprotein I (B2GPI), as pathogenic antibodies. Antibodies to other proteins which have affinity for negatively charged phospholipid have been implicated in the syndrome, including protein C and protein S. The lupus anticoagulant (LA) is an *in vitro* phenomenon in which the antiphospholipid antibody slows clot formation, there by lengthening the clotting time [8]. Whether the association of aPL with thrombosis is causal has been contentious, though studies in experimental animals do suggest that aPL are directly prothrombotic. Many mechanisms for thrombosis in APS have been suggested, such as increased expression of tissue factor on monocytes and endothelial cells, interference in the protein C anticoagulant pathway and inhibition of fibrinolysis. More recently attention has focused on anti-B2GPI [8]. The potential complications of pregnancy in women with antiphospholipid syndrome include recurrent pregnancy loss, preeclampsia, placental insufficiency, maternal thrombosis, and complications due to treatment [9]. The anaemia (Hb < 11.5g/dl) in antiphospholipid women is usually haemolytic in nature, due to autoimmune antibody, with positive direct Coomb's test. Approximately 20 to 40 percent of patients with aPL syndrome have varying degrees of thrombocytopenia platelets count less than $150 \times 10^9/L$ [10]. The treatments of antiphospholipid syndrome include aspirin, heparin, steroids, and immunoglobulins. The latter two have been widely disregarded [5].

MATERIALS AND METHODS

Methods of data collection

This descriptive study was undertaken in Wad Medani Obstetric and Gynecological Hospital, Sudan from August 2012 to August 2013. This hospital is a tertiary referral hospital delivering approximately 4,000 patients per annum. Institutional research and ethics approval was obtained before commencement of the study. All participants were antenatal and post miscarriage patients who spoke sufficient Arabic to provide informed consent. Questionnaires were filled from fifty consecutive patients with a history of unexplained recurrent abortion, defined as spontaneous fetal loss before 24 weeks gestational age, and the cause is not found by the available investigations. Each one of the participants was tested for the presence of lupus anticoagulant (LA), complete blood count (CBC) and both IgG and IgM anticardiolipin (aCL) antibodies. LA and CBC were estimated by automated coagulometer, and automated haematological analyzer respectively and (aCL) was measured using quantitative ELISA.

RESULTS

The results of the analysis of the 50 patients' (5 pregnant and 45 post miscarriages) with a history of unexplained recurrent abortion are as the following: The study includes 45 post miscarriage and 5 pregnant ladies (Fig. 1). The distribution of the age of the study

participants were between 20 – 45 years with the mean of 32 ± 3.4 years. The education levels among the participants varied between 4 years to 17 years with the mean of 13 ± 2.3 years. There is consanguinity relationship in 28 out of the 50 participants and their couples (Fig. 2). The number of miscarriages among the study participants ranging from 3 to 7, they were divided in the following groups: 3 miscarriages include 16 participants. 4 miscarriages include 14 participants. 5 miscarriages or more include 20 participants. No history of thrombosis, pregnancy induced hyper tension, bleeding or skin rash among the participants except one participant with history of skin rash. There were 4 out of 50 participants on oral contraceptive pills at the time of sample taking. There was family history of recurrent abortion in 10 out of the 50 participants (Fig. 3). 49 participants had ideal body mass index (19 to 25), while one participant over weight her body mass index is 26. 10 participants had anaemia (Hb < 11.5gm/dl) [8] with evidence of iron deficiency; no one is proved to be a haemolytic anaemia, while 40 participants had normal haemoglobin levels (Fig. 4). 2 participants had thrombocytopenia (platelets $< 150 \times 10^9/L$),⁽⁸⁾ while 48 had normal platelets counts (Fig. 5). 7 participants fulfill the positive criteria for lupus anticoagulant, while 43 were not (Fig. 6). The serum levels of anticardiolipin antibodies (both IgG and IgM) among the participants ranging from 0.1 to 18.6 they were divided into the following groups: serum levels less than 12 (normal) included 39 participants. Serum levels 12 or more (positive) included 11 participants (Fig. 7). The total positive cases of antiphospholipid antibodies were 13 (5 by both LA and aCL antibodies, 2 by LA alone and 6 by aCL antibodies alone). There was statistically significant negative association between Antiphospholipid antibodies and pregnancy with P value = 0.000 and Chi-Square = 1.952. There was statistically significant association between Antiphospholipid antibodies and the use of oral contraceptive pills with P value = 0.020 and Chi-Square = 5.426.

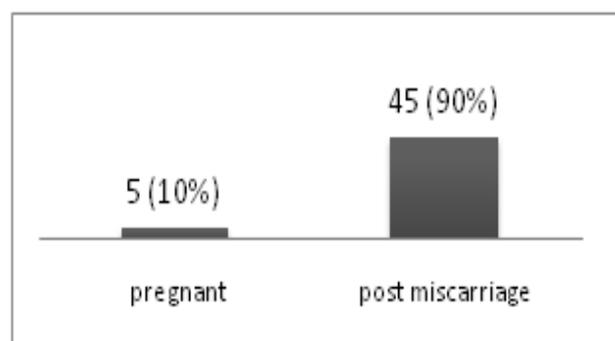


Fig. 1: Post miscarriage and pregnant ladies distribution

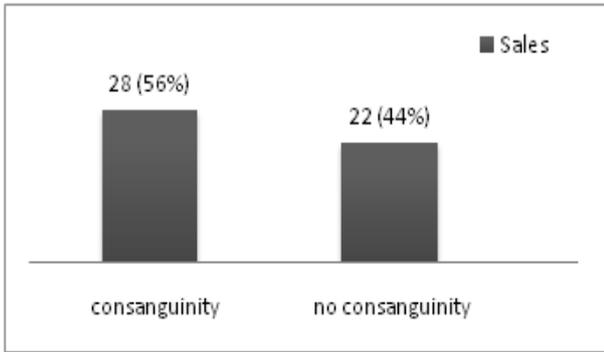


Fig. 2: Consanguinity distributions

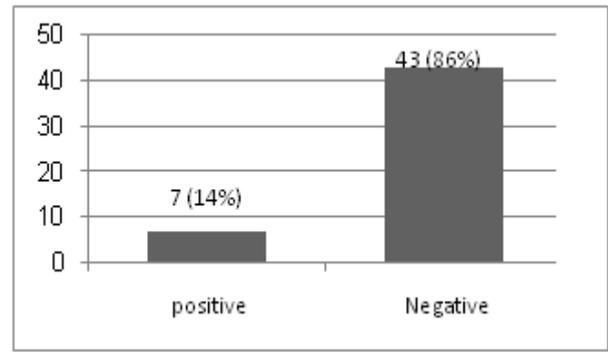


Fig. 6 Lupus anticoagulant test result

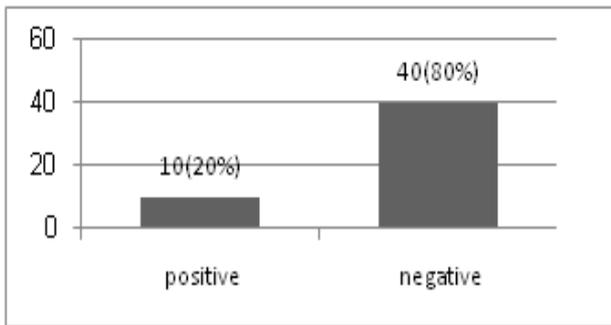


Fig. 3 Family history of recurrent abortion

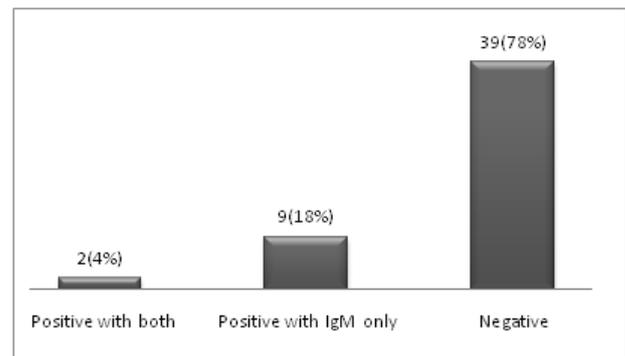


Fig. 7: IgG and IgM aCL antibodies results

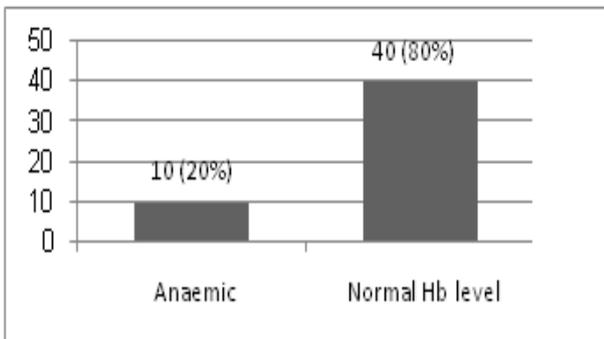


Fig. 4: Distribution of anaemia

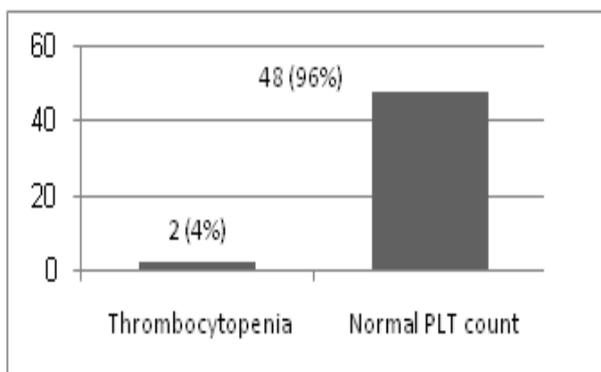


Fig. 5: Distribution of thrombocytopenia

DISCUSSION

Recurrent miscarriage affects 2–5% of the population [1] and because its aetiology often remains unsolved, it is a tragic event for both partners. Recurrent miscarriage is defined as three or more consecutive spontaneous abortions before 20 weeks' gestation. However, in recent articles, a definition of two or more spontaneous abortions is used, because doctors and couples tend to investigate sooner for potential causes [10]. The 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, & anatomic abnormalities [3]. Pregnancy leads to a hypercoagulable state with consequent increased risks of thromboembolism and disseminated intravascular coagulation [6]. Antiphospholipid (aPL) syndrome (APS) represents a significant cause of recurrent miscarriage, it's an acquired thrombophilic disorder in which patients have vascular thrombosis and/or pregnancy complications attributable to placental insufficiency, accompanied by laboratory evidence for the presence of antiphospholipid antibodies in blood. The aim of this study was to estimate the prevalence of antiphospholipid syndrome and to assess the risk factors for recurrent fetal losses in women with unexplained recurrent miscarriage. To carry out this study 50 Sudanese women were enrolled, 5 pregnant and 45 post miscarriage. The prevalence of antiphospholipid syndrome was found to be 13 out of 50 (26%) cases using both anticardiolipin antibodies and LA, this

finding was consistent with studies done on Khartoum University [13] and United Kingdom [9] they found the prevalence of persistently positive tests for aPLs in women with unexplained recurrent abortion were around 20% and 15% respectively. However study done in Gezira university [14], found that the prevalence of aPLs was 1.4%, this most probably because they used only IgG anticardiolipin antibody, which affected by handling and storage. In this study about 11 (22%) cases were positive for IgM aCL antibodies and in 6 (12%) out of them it's the single positive test, although it's persistently positive the role of infectious agents which is known to predispose to aCL antibody production like syphilis, HIV, cytomegalovirus, hepatitis-C and malaria [7] (which is endemic in the study area) cannot be excluded. A statistically significant association (P value = 0.004) was noticed between LA and aCL antibodies. This is because aCL enzyme-linked immunosorbent assay (ELISA) typically detects antibodies to B2-glycoprotein I (B2GPI) [10], and LA tests are sensitive to antibodies to B2GPI and also antibodies to prothrombin [10]. Although LA is relatively less sensitive it has stronger association with pregnancy loss, than anticardiolipin antibodies [9]. There was statistically significant negative association (P value = 0.000) between aPL antibodies and pregnancy. This is most probably due to maternal antiphospholipid antibodies down regulation during pregnancy [4]. Also there was statistically significant association (P value = 0.020) between aPL antibodies and the participants on oral contraceptive pills. There was statistically insignificant association (P value = 0.430) between aPL antibodies and thrombocytopenia, probably due to small sample size. But no association detected with autoimmune haemolytic anaemia apart from one case which has mild anaemia (Hb = 11 g/dl), thrombocytopenia and unconjugated hyperbilirubinaemia (2.5mg/dl) with negative direct Coomb's test. This case by further investigations proved to be a case of systemic lupus erythematosis (SLE). There was high and low association between iron deficiency anaemia and thrombocytopenia respectively and recurrent miscarriage. The anaemia most probably due to repeated blood loss but causative effect cannot be excluded. Also there was high association between couples consanguinity relationships and recurrent miscarriage, but the consanguinity is common in the community. However it increases the chance of chromosomal abnormality which is the main cause of recurrent miscarriage [3]. There was no (or minimal) association with extreme of body mass index (obesity or emaciation) and recurrent miscarriage.

CONCLUSION

The study findings demonstrate that APLS is one of the common causes of recurrent unexplained miscarriage. The results showed that there was statistically significant (100%) clustering of aPL antibodies positivity in participants tested in post miscarriage period. A statistically significant

association was noticed between LA and aCL antibodies. A statistically significant association was demonstrated between aPL antibodies and oral contraceptive pills. There was high association between consanguinity and recurrent miscarriage. Antiphospholipid antibodies positive cases associations revealed statistically insignificant associations with anaemia, thrombocytopenia, education level, consanguinity and body mass index.

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REFERENCES

1. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE *et al.*: Incidence of early loss of pregnancy. *N Engl J Med.*, 1988; 319(4): 189-194.
2. Women Natural Health Practice; Recurrent miscarriage and failed IVF. Available from http://www.naturalgynae.com/downloads/nav6_fact25.pdf
3. Dhont M; Recurrent Miscarriage. *Curr Womens Health Rep.*, 2003; 3(5): 361-366.
4. British Committee for Standards in Haematology; Guidelines on investigation and management of thrombophilia. *J Clin Pathol.*, 1990; 43(9): 703-709.
5. Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V *et al.*; Inherited thrombophilia. Part I. *Thromb Haemost.*, 1996; 76: (5)651-62.
6. Haverkate F, Samama M; Laboratory Investigation of Thrombophilia. Familial dysfibrinogenemia and thrombophilia. *Thromb Haemost.*, 1995; 73(1):151-161.
7. Kaushansky K, Lichtman MA; Williams Hematology. 8th edition, USA: The McGraw-Hill Companies, Inc; 2010.
8. Hoffbrand AV, Catovsky D, Tuddenham EGD; Postgraduate Haematology. 6th edition, Oxford: Wiley & Blackwell Publishing Ltd., 2011.
9. Keeling D, Mackie I, Moore GW, Greer IA, Greaves M, British Committee for Standards in Haematology; Guidelines on the investigation and management of antiphospholipid syndrome. *Br J Haematol.*, 2012; 157(1): 47-58.
10. Branch W, Khamashta MA; Antiphospholipid Syndrome: Obstetric Diagnosis, Management, and Controversies. *Obstet Gynecol.*, 2003; 101(6):1333-1344.

11. Lichtman MA, Williams WJ; Williams Haematology. 7th edition, USA: McGraw-Hill Companies Publishing Ltd., 2007.
12. A Vashisht, Regan L; Antiphospholipid syndrome in pregnancy-an update. J R Coll Physicians Edition, 2005; 35: 337–339.
13. A Bubaker Abd Allah Ahmed; Prevalence of antiphospholipid syndrome among female with recurrent abortion. Obstetric Department, Khartoum University, 2001.
14. Shogar M; Trace minerals and antiphospholipid syndrome in recurrent abortion. University of Gezira, 2013.