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Assessment of selective auto antibodies in pregnant women with spontaneous recurrent miscarriage and non-miscarriage in al- gezira state-sudan

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Abstract:

Recurrent miscarriage is a critical problem in the health sector. Antiphospholipid, anticardiolipin, antinuclear and antidouble strand DNA are group of autoantibodies that bind to negatively charged phospholipids which has relation with recurrent miscarriage during the first 10 weeks of pregnancy or the birth of a dead child (still birth). The aim of this study was to detect the presence of these antibodies among pregnant women in Al-Gezira state. A case-controls study was conducted at Wad Madani Teaching Hospital Al- Gezira, Department of Obstetrics gynecological in Sudan. The cases were (90 women) 45 women with miscarriage while 45 non-miscarriage women chosen as control. Antiphospholipid, Anticardiolipin, Antinuclear and Antidouble strand DNA for IgG antibodies were analyzed in the maternal sera of all of the participants by using ELISA. Furthermore all positive and borderline results were confirmed by using immunoblot test. Out of 90 women, 45 women with miscarriage showed 2(4.4%) were positive, 2(4.4%) were negative for IgG APA 1(2.2%), 2(4.4%) were positive and borderline ACL IgG, 3(6.7%), 1(2.2%) were positive and borderline ANA IgG and negative in dsDNA while 45 women with non-miscarriage 1(2.2%) was borderline APL IgG, 1(2.2%)was borderline ANA IgG and negative in ACL and dsDNA . There were significant difference in the age, biomass index, MCV, MCHC, MPV, RDWCV, RDWSD between the two groups ($P < 0.05$) .The analysis of In binary logistic regression women with variable, univariate and multivariate analysis showed that preeclampsia, vaginal diseases, vaginal bleeding, menstruation cycle, RDWC, RDWSD and biomass index were significantly associated with miscarriage in both ($P < 0.05$). While age were significant associated with miscarriage in univariate analysis (OR= 5, 95%Cl=2-13, P value 0.001), family history (OR= 2.94, 95%Cl=2.946-948, P value.000) and antiphospholipid antibodies associated with miscarriage in multivariate miscarriage (OR= 4.4, 95%Cl=45-43.8, P value0.02). In the current study antiphospholipid, anticardiolipin and antinuclear IgG sero-positivity are associated with miscarriage.

Keywords: Recurrent miscarriage, Antiphospholipid, Anticardiolipin, Antidouble strand DNA, Antinuclear

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Introduction:

Recurrent miscarriage is defined by two or more failed clinical pregnancies and about 50% of reason of recurrent abortion is not clear, and there is increasing evidence that immunological factors play an important role in the failure of these pregnancies [1-3]. These factors include various humoral abnormalities such as anti-nuclear

antibodies (ANA), anti-double stranded DNA antibodies (anti-dsDNA), lupus anti-coagulant (LACAb), anti-phospholipid (APA), anti-cardiolipin (ACA), anti-thyroglobulin (TgAb), anti-thyroperoxidase (TPOAb) and anti-thrombin III antibodies (ATIIIAb). Therefore, in the case of autoimmune-induced miscarriages the woman's body attacks the growing fetus or prevents normal pregnancy progression [4].Further research also has suggested that autoimmune disease may cause genetic abnormalities in embryos, which in turn may lead to miscarriage [5]. Since its original description almost twenty years ago, the antiphospholipid syndrome (APS) has emerged as the most important treatable cause of recurrent miscarriage. The APS is also an important cause of early onset preeclampsia and of IUGR (6). Approximately 15% of women with recurrent miscarriage have persistently positive tests for either LA or ACA, compared to 2% of those with an uncomplicated obstetric history and hence it is important to screen for both LA and for lgG and lgM ACA [7]. Antiphospholipid antibodies are a family of closely related immunoglobulin's that react with anionic phospholipids. Recent studies would suggest these antibodies recognize complexes of anionic phospholipids and a variety of plasma proteins, including 13z-glycoprotein I (~z-GPI), prothrombin, and proteins C and S [7], Anticardiolipin antibodies and lupus anticoagulants are members of the APA family. These antibodies have been associated with thrombotic events, which could lead to pregnancy loss [8]. Clinical features such as venous or arterial thrombosis, unexplained fetal deaths after 10 weeks, delivery at 34 weeks as a result of severe pregnancy-induced hypertension (PIH) or recurrent pregnancy loss (RPL) before 10 weeks in combination with a positive laboratory results of antibodies to cardiolipin (CL) or a positive lupus anticoagulant (LAC) on two occasions at least 6 weeks apart are parameters for diagnosis of the antiphospholipid antibody syndrome (APS) [9]. However, the significance of APA in a woman without of a prior pregnancy or in the absence of prior thromboembolic phenomena is unclear [10]. The presence of an antiphospholipid antibody such as the lupus anticoagulant and anticardiolipin antibodies in an individual is associated with a predisposition for blood clots. Blood clots can form anywhere in the body and can lead to stroke, gangrene, heart attack, and other serious complications. Antiphosholipid and anticardiolipin antibodies are frequently found in sera of patients with systemic lupus erythematous (SLE) and related diseases or in patients without any evidence of autoimmune disorders [11, 12].These antibodies have been shown to be associated with thrombosis, thrombocytopenia, and recurrent fetal loss, which together comprise the antiphospholipid syndrome APS) [13, 14]. However, a few studies have been conducted in the Sudan about causative agents of miscarriage as case control studies to determine the association between the autoantibodies and recurrent fetal loss.

Methods

A case – control study was conducted at Wad Madani Teaching Hospital, Al-Gezira, Sudan during the period of August through November 2018. Including 90 (100%) participants women with and without history of miscarriage were enrolled, 45(50%) women were pregnant with history of miscarriage, while the others 45(50%) were pregnant without history of miscarriage, five ml of blood specimens were collected from each participated women and dispensed into sterile EDTA and plain blood containers, then serum samples were extracted from blood of women by centrifugation at 3000 for 5 minutes. Enzyme-linked immunosorbent assay (ELISA) was used for antiphospholipid, anticardiolipin, antinuclear and Antidouble strand DNA for (IgG) using commercial diagnostic kits (AESKU, Germany). Quantitative analysis for APL, ACL, ANA, dsDNA (IgG) were performed, and the assay result interpreted as positive more than (1.2 × OD cut-off), borderline (0.8 × OD cut-off) ≤ OD patient ≤ (1.2 × OD cut-off), negative less than (0.8 × OD cut-off). Finally, all positive and borderline results were confirmed by using immunoblot test using commercial diagnostic kits (AESKU). Antibodies were detected using EUROIMMUN immunoblot strips coated with parallel lines of highly purified antigens. The EUROLINE test kit provides a qualitative *in vitro* assay for human autoantibodies of the IgG class to 14 different antigens (n-RNP, Sm, SS-A native and RO52, SS-B, Scl70, PM-Scl, JO-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal-P-protein, and AMA M2). 1.5ml of the diluted enzyme conjugate (alkaline phosphate labeled ant human IgG) were pipetted into each channel, and incubated for 30 minutes at RT on a rocking shaker, afterwards this liquid was aspirated off. A Second wash was carried out 5X with 5 minutes interval with 1.5ml working strength wash-buffer per a strip, on a rocking shaker, then 1.5 ml of a substrate solution were aspirated down into the channels of the incubation trays the trays were covered with clean papers and incubated for 10 minutes at RT on a rocking shaker. After the substrate solution was aspirated off, each strip was washed with distilled water as 3times 1minutes. The strips were left to air dry then were evaluated using the EUROIMMUN sheet.

Statistical analyses

Data was analyzed by statistical package for social sciences (SPSS) program version 21 for interpretation of the results and binary regression analyses, logistic regression analysis test was employed to assess the association between variables. Odds ratio (OR) with 95% confidence interval (CI) was calculated and statistical significance was set at $P < 0.05$. Frequencies, proportion were drawn for numerical data and recurrent response was done for quantitative data mean and standard deviation.

Results

A total of 90 participants women were enrolled to the study there were 45 cases and 45 controls, table (1) showed that the occurrence of APA ,ACL, ANA, Ads IgG between two groups, there are 2(4.4%) positive, 2(4.4%) borderline and

41(91.1%) negative for APL-IgG, 1(2.2%) positive, 2(4.4%) borderline and 42(93.3%) negative for ACL-IgG, 3(6.7%) positive, 1(2.2%) borderline and 41(91.1%) negative for ANA-IgG, while dsDNA-IgG was 0(0.0%) positive and borderline, 45(100%) negative cases of women with recurrent miscarriage. And IgG for non-miscarriage women was 0(0.0%) positive, 1(2.2%) borderline and 44(97.8%) negative for APL and ANA antibodies, 0(0.0%) positive and borderline, 45(100%) negative for ACL and dsDNA antibodies.

Table 1: Distribution of women with recurrent miscarriage and non-miscarriage according to Antiphospholipid, Anticardiolipin, Antinuclear and Antidouble strand DNA antibodies for IgG:

Item	Women with recurrent miscarriage	Women with non-miscarriage	Total
Antiphospholipid Ab	Positive 2(4.4%) Borderline 2(4.4%) Negative 41(91.1%)	0(0.0%) 1(2.2%) 44(97.8%)	2 3 85
Total	45	45	90
Anticardiolipin Ab	Positive 1(2.2%) Borderline 2 (4.4%) Negative 42(93.3%)	0(0.0%) 0(0.0%) 45(100%)	1 2 87
Total	45	45	90
Antinuclear Ab	Positive 3(6.7%) Borderline 1(2.2%) Negative 41(91.1%)	0(0.0%) 1(2.2%) 44(97.8)	3 2 85
Total	45	45	90
Antidouble strand Ab	Positive 0(0.0%) Borderline 0(0.0%) Negative 45(100%)	0(0.0%) 0(0.0%) 45(100%)	0 0 90
Total	45	45	90

Table2: Confirm positive and borderline results by using immunoblot test:

SNO	**Antibody**	**Diseases**
86	CENP-B	Systemic sclerosis
55	SS-A/RO60 +SS-A/RO52	Sjorgen's syndrome
39	PCNA	Systemic lupus erythematous
75	dsDNA + CENP-B +AMA M2	Systemic lupus erythematous
77	dsDNA + PCNA+ SS-B	Systemic lupus erythematous
61	AMA	Primary biliary cirrhosis

Socio-demographic and clinical characteristics with (p value < 0.05) include: age (P= 0.0003), Biomass index (P=0.0104), MCV (P=0.0001), MCHC (P=0.0125), MPV (P=0.0006), RDWCV (P=0.0044), RDWSD (P=0.0001). While there was no significant difference between case and control include Hb, RBCs, TWBCs, Platelet, PCV, MCH, Antiphospholipid IgG, Anticardiolipid IgG with means as showed in Table (3).

Table 3: Socio-demographical and clinical characteristic of the cases and controls.

Variable	Control N=45 Mean ± SEM	Case N=45 Mean ± SEM	P-value
Age	26.02 ± 0.8531	30.89 ± 0.9504	0.0003 "-7.409 to -2.324
Biomass index	25.66 ± 0.6089	27.85 ± 0.5751	0.0104 "-3.860 to -0.5250
Hb	10.93 ± 0.2420	10.58 ± 0.3481	0.4187 "-0.4995 to 1.188
RBCs	10.65 ± 6.849	3.843 ± 0.1349	0.3235 "-6.836 to 20.44
TWBCs	9.109 ± 0.4661	7.907 ± 1.214	0.3577 "-1.386 to 3.790"
Platelates	251.7 ± 12.61	243.8 ± 14.61	0.6803 "-30.43 to 46.39"
PCV	33.94 ± 0.6871	31.84 ± 1.053	0.0984 "-0.4025 to 4.602
MCV	90.72 ± 1.057	84.22 ± 1.010	0.0001 "3.590 to 9.410
MCH	29.00 ± 0.5027	28.11 ± 0.5391	0.2311 "-0.5784 to 2.356
MCHC	31.91 ± 0.3579	33.16 ± 0.3316	0.0125 "-2.216 to -0.2733
MPV	8.687 ± 0.1015	9.593 ± 0.2327	0.0006 "-1.412 to -0.4012
PCT	0.2115 ± 0.01113	0.2579 ± 0.03219	0.1762-0.1143 to 0.02136
RDWCV	15.88 ± 0.2821	14.59 ± 0.3397	0.0044 "0.4121 to 2.170
RDWSD	52.48 ± 0.8195	44.98 ± 0.8974	0.0001 "5.078 to 9.917

RBCs (Red blood cells), Hb (Hemoglobin), PCV(Packed Cell Volume),MCV (Mean Cell Volume), MCH (Mean cell hemoglobin), MPV (Mean Platelet Volume), PCT (Plateletcrit), RDW-CV (Red Blood Cell Distribution Width), RDW-SD (Red Cell distribution width it measures the width of red cells size distribution).

In binary logistic regression women with variable, univariate and multivariate analysis showed that preeclampsia, vaginal bleeding, menstruation cycle, RDWC, RDWSD and biomass index were significantly associated with miscarriage in both univariate and multivariate. While age were significant associated with miscarriage in univariate analysis (OR = 5, 95% Cl = 2 -13, P value 0.001), family history (OR = 2.94, 95% Cl = 2.946 - 948, P value.000) and antiphospholipid antibodies associated with miscarriage in multivariate (OR = 4.4, 95% Cl = 45 - 43.8, P value0.02). Table (4).

Table 4: Binary regression analyses of the predictors for miscarriage and non-miscarriage:

Variable	Univariate			Multivariate		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Age	5	2-13	0.001	.336	.090-1.250	.104
Biomass index	5	2-12	0.001	1.73	0.062-4.79	0.001
Rate of Miscarriage	.000	0.000-0.000	0.993	1.000E-013	0.000-1.000	0.9987
Family history	2.94	2.946-948	.000	1.000	0.000-1.000	1.000
Menstruation Cycle	3.775	1.2-11.5	0.02	2.59	0.078-8.61	0.028
Vaginal disease	0.230	0.211-1.453	0.230	0.689	0.239-1.987	.491
Vaginal Bleeding	6.353	2.1-19.2	.001	1.39	.043-4.47	0.001
Hypertension	1.08	0.065-17.8	0.96	0.972	0.057-15.741	0.951
Preeclampsia	16.1	1.9-131.1	0.01	2.9	1.3 -6.7	0.000
Blood group	0.000	.010-1.722	0.1	7.2	5.4-8.42	0.997
HB	1.3	0.56-3.1	0.4	0.574	0.126-2.615	.473
RBCs	2.3	0.85-6.2	0.1	0.494	0.156-1.564	0.230
TWBCS	0.7	0.254-1.97	0.5	1.928	0.635-5.855	.246
Platelets	0.7	0.21-2.2	0.52	1.208	0.343-4.251	.768
PCV	.432	0.162-1.157	.095	.594	0.180-1.959	.392
MCV	1.000	0.234-4.271	1.000	1.571	.327-7.549	.573
MCH	.577	0.248-1.343	.202	0.442	0.165-1.188	0.106
MCHC	1.545	0.616-3.878	.354	2.112	0.759-5.881	.152
MPV	.302	.058-1.587	.157	0.677	0.085-5.401	0.713
PCT	1.000	0.269-3.724	1.000	0.811	0.144-4.576	0.813
RDWCV	4.375	1.750-10.9	.002	3.531	1.190-10.472	.023
RDWSD	19.158	5.158-71.1	.000	17.019	4.187-69.179	.000
Antiphospholipid IgG	43	.46-40.01	.2	4.4	.45-43.8	.02
Anticardiolipid IgG	1	1-1	0.748	0.57	.007-.460	.017
Antinuclear IgG	0.9	0.56-15.9	0.9	1.170	.06-23.6	0.9
Antidoublestrand DNA IgG			1.000	0.063	0.01-0.5	.017

Cl: Confidence interval , OR: Odds ration RBCs(Red blood cells),Hb (Hemoglobin), RBCs (Red Blood ell), TWBCs(Total White blood cells),PCV(Packed Cell Volume),MCV(Mean Cell Volume),MCH (Mean cell hemoglobin),MPV (Mean Platelet Volume), PCT (Plateletcrit),RDW-CV (Red Blood Cell Distribution Width),RDW-SD(Red Cell distribution width it measures the width of red cells size distribution)

Discussion:

In this study women with fetal loss had relatively high incidence of raised antiphospholipid antibodies, anticardiolipin antibodies and antinuclear antibodies of the IgG class Table (1), and three of them were diagnosed with Systemic lupus erythematous (SLE), Table (2) a finding which accordance with previous reports. Presence of clinical features previously associated with raised antiphospholipid, anticardiolipin, antinuclear antibodies levels [15].

Our study revealed there was no statistically significant difference in HB, platelets. While the age, (control) group were N = 45 Mean 26.02 and (case) group were N = 45 mean 30.89, this result showed statistical significant (P < 0.0003) between control and case group. The age of most positive cases in miscarriage women was belonged to more than 30, this result agrees with [16],who found that women of (31-35) years had a 28% miscarriage rate. Because the causative agents of miscarriage are many reasons, still need further studies. In the present study in all participants the biomass-index seropositivity of APL, ACL, and ANA-IgG were found higher in abnormal participant, the results that agreed with previous studies, who established there were strong associations between abdominal obesity and seropositivity in women participating. Interestingly, according to our study revealed many factors related to the biomass-index the (case) group was N=45 mean 25.66 and (control) group was N = 45 mean 27.85 this result showed statistical significant (P = 0.0104) and another factors as remarkable signs of MCV (P=0.0001), MCHC (P=0.0125), MPV (P=0.0006), RDWCV (P=0.0044), RDWSD (P=0.0001) Table (3).

In binary logistic regression women with variable, univariate and multivariate analysis showed that preeclampsia, microcytic hypo- chromic anemia, vaginal bleeding, menstruation cycle, RDWC, RDWSD and biomass index were significantly associated with miscarriage in both univariate and multivariate. While age were significant associated with miscarriage in univariate analysis, antiphospholipid antibodies associated with miscarriage in multivariate (OR= 4.4, 95%Cl=45-43.8, P value0.02). On the other hand, the results revealed that was statistical relation between the disease and the family history (OR= 2.94, 95%Cl=2.946-948, P value.000), this result agreed with that of [17] who found about 60% of early pregnancy losses associated with sporadic chromosomal anomalies. Table (4).

The high level of antibodies is known to be harmful to both mother and child, so diagnosis and treatment can improve pregnancy outcome [18]. In these study, Presence of clinical features previously associated with raised anticardiolipin antibody levels: thrombosis (both venous and arterial), [15] fetal loss, [19]and thrombocytopenia [20] confirms the validity of our study. In our study presented that there are many risk factors associated with recurrent miscarriage.

Many studies agreed with our results that found significant association between repeated miscarriage and presence of high level of APA

ANA and ACL- Abs of clinical significance in identifying women at a risk of pregnancy loss then ACL-IgG antibodies. In different studies there was high a variation in rate of APA, ACL-Abs. which could be due to many causes of repeated abortion in women and the different methods that employed to determinate antibodies so, these results indicated the importance of systemic investigation of these antibodies in pregnant women at risk of miscarriage, and Identification of patients positive for antiphospholipid antibodies. it is importance to record. However, that several reports had described successful pregnancies in patients with antiphospholipid antibodies, treated during pregnancy [21, 22]. Whether antiphospholipid antibodies are causative, a consequence or coincidence remains unknown but there is general agreement that the pregnant woman is particularly vulnerable to the adverse effects associated with these antibodies [23]. Limitation of our study needs increasing of sample size and cohort study design to follow up pregnant women and further studies on molecular and cytokines levels.

Conclusion:

The current study revealed that seroprevalence rate in women with fetal loss there was a relatively high incidence of raised antiphospholipid antibodies, anticardiolipin antibodies and antinuclear antibodies of the IgG class and three of them were Systemic lupus erythematous (SLE) patients. Also there was a significant association between menstruation cycle preeclampsia, vaginal disease, vaginal bleeding and family history with recurrent miscarriage.

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