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Antiphospholipid Syndrome And Triple Antibodies Profiling Among Pregnant Women With Complications In A Tertiary Health Institution In Southwest Nigeria

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Abstract: ***Background: The prevalence of Antiphospholipid Syndrome (APS) which increases with age and obstetric complications are believed to be under estimated in this environment due to the use of single or double antiphosholipids antibodies for its determination.***

Aim: To determine the pattern of distribution of IgG and IgM of anti-cardiolipins antibodies and lupus anticoagulant among pregnant women in LAUTECH Teaching Hospital Ogbomoso.

Method: One hundred and sixty participants were administered questionnaires. Eighty pregnant women with complications in previous pregnancy as study subjects and those without complications as controls. Blood specimens were collected for the estimation of human anticardiolipin IgG and IgM and the human lupus anticoagulant using Enzyme Linked Immunosorbent Assay kits for the detection of antibodies to cardiolipin and lupus anticoagulant.

Result: The prevalence of positivity to IgG anti-cardiolipins was significantly higher in the study group (25%) compared to the controls (7.5%), while positivity to IgM anti-cardiolipins in the study group (28.8%) compared to the controls (6.2%). Positivity to Lupus anti-coagulants among participants in the study group differed significantly compared to the controls (17.5% vs 5%).

After twelve weeks of re-assessment, seroconversion occurred in all participants in the control group with prior positivity at first contact to IgG anti-cardiolipins and fourteen (17.5%) participants were persistently positive. There was a statistical significant difference in persistent positivity to IgM anti-cardiolipins between the controls (2.5%), and the study group (21.3%). The prevalence of persistent positivity to Lupus anti-coagulant was significantly higher in the study group (16.3%) compared with the controls (2.5%).

Conclusion: The prevalence of APS among pregnant women with complications was 28.8% compared with prevalence of 2.5% among healthy pregnant women.

Keywords: Antiphospholipid Syndrome; intra-uterine growth restriction; thrombocytopenia; anti-cardiolipins antibodies; lupus anticoagulant.

I. INTRODUCTION

Antiphospholipid Syndrome (APS) is an autoimmune disease characterized by clinical thrombotic events which may be arterial or venous vasculature-associated with the presence of antiphospholipid antibodies in patient's plasma.[1], [2] It was first described in early 1980s by Hughes, Harris and Gharavi as an acquired thrombophilic disorder in which

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autoantibodies are produced to a variety of phospholipids and phospholipids binding proteins.[1]-[4] Since its first description in early 1980s, several cases have been reported from Africa and other parts of the world.[2], [4] The core features that are associated with this syndrome include arterial and venous thromboses, recurrent spontaneous miscarriages, preeclampsia, Intra-uterine growth restriction (IUGR), thrombocytopenia and the presence of high titres of antiphospholipid antibodies (aPLs) in the blood of the subject. [2], [4], [6] The precise mechanism whereby haemostasis is altered to induce a hypercoagulable state in APS is unclear and several mechanisms are responsible for clinical manifestations in patients who have this syndrome. [7], [8]

The prevalence of APS which increases with age and obstetric complications are believed to be the result of different mechanisms acting on the placental cells and the endometrial tissues. Thrombosis, inflammation and immunomodulation are also thought to play a role. [11]-[13] Pertinent antiphospholipids implicated in APS include anticardiolipins, beta2 glycoprotein and lupus anticoagulant but there are also clinical conditions with seronegativity. [11], [14], [16] These antiphospholipids constitute the triple antibodies which are currently being used in profiling for the diagnosis of primary and secondary APS.

Antiphospholipid syndrome is sometimes overlooked in many part of the world especially in developing countries and it is an acquired systemic autoimmune disease characterized by thrombosis and pregnancy morbidity in the presence of persistently positive antibodies. [1], [16], [20] The most commonly detected antiphospholipid antibodies are lupus anticoagulant, anticardiolipin and anti-beta 2 GPI antibodies which are detected by immunoassays that measure the immunogenic reactivity to a phospholipid or a phospholipid-binding protein.

Like other autoimmune disorders, APS does not have a known aetiology but there are several hypotheses to explain the probable cause. These include passive transfer of maternal autoantibodies to the foetus, and familial occurrence of antiphospholipids with genetic association like HLA-DR4, DR7, DRw53 and C4 null allele. [3], [16]

Several studies from different parts of the world have established the prevalence of anti-phospholipid antibodies in the healthy general population to be around 1-5% by using presence of triple antibodies using either lupus anticoagulant, anticardiolipins and beta 2 glycoprotein I antibodies. [4], [19] The relationship of these autoantibodies and pregnancy morbidity accounts for approximately 15% of cases and warranted further research in different isotypes of these antibodies. [5] Also, the risk of a first thrombotic event among asymptomatic persons who are positive for lupus anticoagulant, anticardiolipin and anti-beta 2 glycoprotein I antibodies is 5.3% per year and several studies have associated antiphospholipid antibodies with miscarriages, preeclampsia and intrauterine growth restriction with few local studies which only made use of single or double antibodies. [17], [5] Most studies in Nigeria found the prevalence of antiphospholipid syndrome among pregnant women using lupus anticoagulant and IgG anticardiolipin to range between 2.3-8.2%. [17], [6]

However, studies of these triple autoantibodies concomitantly in this part of the world are very rare. These antiphospholipids antibodies are strong factors in several pregnancy complications and the pattern and distribution of each isotype in relation to different pregnancy complications differ from region to region.

Therefore, this study was designed to determine the pattern of distribution of IgG and IgM of anti-cardiolipins antibodies and lupus anticoagulant among pregnant women in LAUTECH Teaching Hospital Ogbomoso.

More so, the study of triple antibodies in this environment will help in identifying symptomatic and asymptomatic patients who could later develop one or more complications in late pregnancy. Introducing the test for triple antibodies in routine screening of antenatal patient will serve as an invaluable tool in the early diagnosis of asymptomatic patient which will facilitate prompt treatment plan to prevent complications that may arise and this will also help in preconception management of such patient in the future.

II. MATERIALS AND METHODS

A. STUDY POPULATION

This was a longitudinal descriptive study that was carried out in Ladoke Akintola University Teaching Hospital, Ogbomoso. The study population consists of eighty eligible consented pregnant women with previous pregnancy complications, who were consecutively recruited from the Booking Clinic, Antenatal Clinic, Gynaecological Emergency Unit and Labour Ward of the hospital with eighty age-matched eligible consented healthy pregnant women as controls. The subjects were pregnant women in second trimester of pregnancy whose pregnancy was confirmed by serum human chorionic gonadotropin assay or by ultrasonography with past history of ≥2 consecutive spontaneous abortions, past history of previous unexplained intrauterine fetal death, history of previous preterm delivery and people with systolic blood pressure ≥140mmHg and diastolic blood pressure of ≥90mmHg in the index pregnancy. The same number of healthy pregnant controls without history of pregnancy complications and not having any autoimmune or any medical disease, history of thromboembolic, and had at least one live birth were recruited.

A total number of one hundred and sixty questionnaires were administered, eighty each for study subjects and controls. Institutional Ethical Approval was obtained for this study. Participants gave their written consent and their results were made available to their managing Obstetrician for their adequate management.

B. SAMPLE COLLECTION

Ten millimeters of blood was collected from the antecubital vein of each of the participant on two occasions (at least 12 weeks' interval), 4.5mls was dispensed into a sample bottle containing 0.5mls of 3.2% (0.109M) trisodium citrate anticoagulant. The citrated samples were centrifuged at 1500rpm for 15 minutes and the supernatant (platelet poor

plasma) was separated into a plain tube and stored in -20°C freezer. The citrated sample was used for Lupus Anticoagulant (LA) assay. Another 5.5mls of blood was dispensed into a plain container, allowed to clot and retracted. The clotted sample was centrifuged at 1500rpm for 10mins and the supernatant (serum) was separated into another plain container and stored in a -20^{0}C freezer until analyzed.

C. LABORATORY ANALYSIS OF BIOCHEMICAL PARAMETERS

The human anticardiolipin IgG and IgM Enzyme Linked Immunosorbent Assay (ELISA) kits and the Human lupus anticoagulant ELISA Kit were used for the detection of IgG and IgM antibodies to cardiolipin and lupus anticoagulant in human serum [9], [10]. Using indirect enzyme immunoassay from SPAN Biotech Limited with lot numbers and reference numbers 2017O9 (E20170929304) ACA 521T; 201709 (E20170929001), ACA 385T; E20170926005 and reference number HLA 503G. The assays were done using Labtech LT-4000 microplate Reader at a wavelength of 450nm.

D. STATISTICAL ANALYSIS

Data entry was analyzed using Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables were expressed in percentages and continuous variables were summarized using mean ± standard deviation. Relationships between categorical variables were analyzed using chi-square, Fisher's test and likelihood ratio test where appropriate. Differences in means between continuous variables were analyzed using t-test. Clinical complications such as miscarriages and hypertension were disaggregated into categories; Miscarriage (<3 / ≥3), Hypertension (Hypertensive / Not Hypertensive). Clinical parameters were used as independent variables, Status of participants to antiphospholipid was used as dependent variable and a binary logistic regression was conducted to examine the effect of clinical factors on the distribution of antibodies. Level of significance was set at 5%.

III. RESULTS

One hundred and sixty questionnaires were administered in all, 80 to participants who had obstetric complications and 80 to apparently healthy pregnant women who served as controls. All the questionnaires were completed and appropriate blood samples collected at first contact and at twelve weeks after, given a total response of 100%.

A. SOCIO DEMOGRAPHIC PROFILE OF PARTICIPANTS

Table 1: below shows the socio demographic profile of the participants. Participants were considered based on age specific fertility rate and the study found that majority of the participants in both groups (Control- 27.50% and Study- 26.25%) were aged between 30-34 years. There was no statistical significant difference in the mean age of control (mean = 29.7 ± 5.2) and the study group (mean = 29.9 ± 4.9), t = -0.25, p-value = 0.803. Preponderant percentages of the participants were Christians (63.75% - control group and 77.5% - Study group), majority of which were married (Control-96.25% and Study-100%) and the dominant ethnic group was Yoruba (93.75% - Control group and 92.5 % - Study group). High level of education was demonstrated within the groups, with majority of participants in both groups found to have tertiary education, chi square statistic = 10.194, p-value = 0.017. Majority of the participants were civil servant (37.5%- Control and 63.75% - Study group).

Characteristics	Control	Study	Test statistic	p-value
Age groups				
15-19 years	7 (8.75)	5 (6.25)	X^2 = 1.473	0.9160
20-24 years	20 (25)	19 (23.75)		
25-29 years	14 (17.5)	17 (21.25)		
30-34 years	22 (27.5)	21 (26.25)	t-statistic =	0.8030
35-39 years	11 (13.75)	9 (11.25)	-0.2500	
40-44 years	6 (7.5)	9 (11.25)		
Mean age ± SD	29.7 ± 5.2	29.9 ± 4.9		
Religion				
Islam	29 (36.25)	17 (21.25)		
Christianity	51 (63.75)	62 (77.5)	5.7600	0.1240
Traditional	-	1 (1.25)		
Marital Status				
Married	77 (96.25)	80 (100)	3.0570	0.0800
Single Mother	3 (3.75)	-		
Years of Marriage (years)	55 (68.75)	58 (72.5)		
1- 5 years	24 (30)	17 (21.25)	4.0750	0.2540
6- 10 years	1 (1.25)	4 (5)		
11 – 15 years	-	1 (1.25)		
Above 15 years				
Ethnic group				
Yoruba	75 (93.75)	74 (92.5)		
Igbo	2 (2.5)	5 (6.25)	2.2920	0.3180
Hausa	-	-		
Others	3 (3.75)	1 (1.25)		
Highest level of education	3 (3.75)	1 (1.25)		
Primary	28 (35)	12 (15)	10.1940	*0.0170
Secondary	3 (3.75)	4 (5)		
Post-Secondary	46 (57.5)	63 (78.75)		
Tertiary				
Occupation				
Civil Servant	30 (37.5)	51 (63.75)		
Trading	22 (27.5)	14 (17.5)	26.6170	*0.0010
Artisan	20 (25)	1 (1.25)		
Student	8 (10)	14 (17.5)		

**p-value < 0.05 indicate significance, x^2 = Chi-square statistic*

Table 1: Socio demographic profile of participants

B. GYNAECOLOGICAL HISTORY OF PARTICIPANTS

Table 2: The mean age at menarche was similar in the controls and the study group (14.3 ±1.6 years' vs 14.1 ± 1.8 years), t = 0.647, p-value = 0.519. No statistical significant difference was observed in the mean cycle length of the controls and the study group (27.5 ± 1.9 days' vs 27.5 ± 2.3 days, t= 0.112, p-value = 0.911). The mean duration of menstrual flow was significantly longer in the study group when compared with controls (Control - 3.74 ±3.236 days' vs Study - 4.23 ± 0.8days, t = -3.263, p-value = 0.002). Spotting between menstrual periods was evident in 5% of women with complications and 15% of healthy pregnant women. Bleeding during intercourse occurred only in three (3.8%) healthy pregnant women. No history of dysmenorrhea among participants associated with group (36.2% - control group vs 27.5% - study group), X^2 = 0.935, p-value = 0.334.

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VARIABLE	n (%) Control group n = 80	n (%) Study group n = 80	Test statistic	p-value
Age at menarche (in years)	25 (31.2)	29 (36.2)		
10 – 13	47 (58.8)	45 (56.2)	$X^2 = 0.625$	0.731
14 – 16	8 (10.0)	6 (7.5)		
≥ 17	14.3 ± 1.6	14.1 ± 1.8	t = 0.647	0.519
Mean ± SD				
Cycle length (Days)				
Mean ± SD	27.5 ± 1.9	27.5 ± 2.3	t = 0.112	0.911
Duration of menstrual flow (Days)	3.74 ± 1.1	4.23 ± 0.8	t = -3.236	*0.002
Mean ± SD				
Spotting between periods				
Yes	12 (15)	4 (5.0)	$X^2 = 4.444$	0.035
No	68 (85.0)	76 (95.0)		
Bleeding during intercourse	3 (3.8)	0 (0.0)	Fisher's	0.245
Yes	77 996.2)	80 (100)	exact test	
No				
Dysmenorrhea				
Yes	27 (36.2)	22 (27.5)	$X^2 = 0.935$	0.334
No	51 (63.8)	58 (72.5)		

**p-value < 0.05 indicate significance, x^2 = Chi-square statistic*

Table 2: Gynaecological parameters of participants

C. OBSTETRICS PARAMETERS OF RESPONDENTS

Table 3: The minimum gestational age of participants among study group was 13 weeks, the maximum of 30 weeks while the minimum gestational age in the control group was 13 weeks and a maximum of 29 weeks. The mean gestational age of participants was significantly higher in the study group compared to the controls (Control - 20.25 ± 4.48 vs Study- 22.65 ± 4.67, t = -3.3180, p-value = 0.0010). The proportion of participants with at least one child born at full term was similar among control (37, 46.2%) and the study group (40, 52.6%), $\chi^2 = 0.10$, p-value = 0.7520. Seven (9.2%) participants in the study group had at least a premature birth; foetal deaths occurred in two (2.5%) participants, foetal growth retardation in one (1.2%) participant and four (5%) participants had experienced severe eclampsia in their previous pregnancies.

VARIABLES	n (%) Control group n = 80	Study group n = 80	Test statistic	p-value
Gestational Age				
Minimum	13	13		
Maximum	29	30		
Mean ± SD	20.25 ± 4.48	22.65 ± 4.67	t = -3.318	*0.001
Miscarriages				
0	0 (0.0)	30 (37.5)		
1 - 2	0 (0.0)	36 (45.0)		
≥3	0 (0.0)	14 (17.5)		
Foetal death				
Yes	0	2 (2.5)		
No	80 (100)	78 (97.5)		
Past history of fetal growth retardation				
Yes	0 (0)	1 (1.2)		
No	80 (100)	79 (98.8)		
Premature Birth				
≥1	0 (0)	7 (9.2)		
Previous Eclampsia or severe pre-eclampsia during pregnancy	0 (0)	4 (5)		
Yes	80 (100)	76 (75)		
No				
Number of child at full term	37 (46.2)	40 (52.6)	$X^2 = 0.10$	0.752
≥1				

Table 3: Obstetric parameters of participant disaggregated by groups

D. ANTHROPOMETRIC MEASUREMENT AND CLINICAL PARAMETERS OF THE PARTICIPANTS

Table 4: The mean height (m) of participants in the control group compared with the study group do not differ significantly (1.61 ± 0.6 vs 1.60 ± 0.6, t= 1.220, p-value = 0.224). The mean weight (kg) of participants in the control group differ significantly from the study group (65.63 ± 12.51 vs 70.32 ± 11.5, t = -2.467, p-value = 0.015). Thirty-three (41.2%) participants in the study group were hypertensive with 30 (37.5%) participants observed to have systolic blood pressure ≥ 140mmHg, and 33 (41.2%) participants had diastolic blood pressure ≥ 90mmHg.

There was a statistical significant difference in the mean systolic blood pressure (109.5 ± 10.5 vs 130.9 ±30.2mmHg, t = -5.982, p-value = 0.001) and mean diastolic blood pressure (68.9 ± 7.4mmHg vs 82.9 ± 19.2, t = -6.101, p-value = 0.001) between the controls and the study group.

VARIABLES	Control group n = 80	Study group n = 80	Test statistics	p-value
Height (meters)				
Mean ± SD	1.61 ± 0.6	1.60 ± 0.6	t = 1.220	0.224
Weight (Kg)				
Mean ± SD	65.63 ± 12.51	70.32 ± 11.5	t = -2.467	*0.015
Systolic BP (mmHg)				
<140	80 (100)	50 (62.5)	$X^2 = 36.9$	*0.001
≥ 140	0 (0.0)	30 (37.5)		
Mean ± SD	109.5 ± 10.5	130.9 ± 30.2	t = -5.982	*0.001
Diastolic BP (mmHg)				
<90	80 (100)	47 (58.8)	$X^2 = 41.6$	*0.001
≥ 90	0 (0.0)	33 (41.2)	t = -6.101	*0.001
Mean ± SD	68.9 ± 7.4	82.9 ± 19.2		
Hypertension status of participants	0 (0.0)	33 (41.2)	41.6	*0.001
Hypertensive	80 (100)	47 (58.8)		
Non-Hypertensive.				

**p-value < 0.05 indicate significance, x^2 = Chi-square statistic*

Table 4: Distribution of participants by anthropometric measurements and clinical parameters

E. PATTERN OF BIOCHEMICAL PARAMETERS AMONG PARTICIPANTS AT FIRST CONTACT

Table 5: depicts the prevalence of each auto-antibody disaggregated by groups. The prevalence of positivity to IgG anti-cardiolipins was significantly higher in the study group (n = 20, 25%) compared to the controls (n = 6, 7.5%), $\chi^2 = 7.76$, df = 1, p-value = 0.005. There was a statistical significant difference in proportion of participants who tested positive to IgM anti-cardiolipins in the study group (n = 23, 28.8%) compared to the controls (n = 5, 6.2%), $\chi^2 = 12.51$, df = 1, p-value = 0.0004. Positivity to Lupus anti-coagulants among participants in the study group differed significantly compared to the controls (n = 14, 17.5% vs n = 4, 5%), $\chi^2 = 5.07$, df = 1, p-value = 0.0240.

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Biochemical parameters	Control n (%)	Study n (%)	χ^2 value	df	p-value
IgG anti-cardiolipins	6 (7.5)	20 (25)	7.76	1	*0.005
IgM anti-cardiolipins	5 (6.2)	23 (28.8)	12.51	1	*0.000
Lupus anti-coagulant	4 (5.0)	14 (17.5)	5.07	1	4 *0.024

**p-value < 0.05 indicate significance, x^2 = Chi-square statistic.*

Table 5: Prevalence of positivity to anti-phospholipids anti-bodies among participants at first contact disaggregated by groups

F. PATTERN OF BIOCHEMICAL PARAMETERS AMONG PARTICIPANTS AFTER TWELVE WEEKS

Table 6: Seroconversion occurred in all participants in the control group with prior positivity at first contact to IgG anti-cardiolipins and fourteen (17.5%) participants were persistently positive in the study group after twelve weeks of re-assessment to estimate the prevalence of persistent positivity to each biochemical parameters.

There was a statistical significant difference in persistent positivity to IgM anti-cardiolipins between the controls (n = 2, 2.5%), and the study group (n = 17, 21.3%), χ^2 = 11.7, df = 1, p-value = 0.0006. The prevalence of persistent positivity to Lupus anti-coagulant was significantly higher in the study group (n = 13, 16.3%) compared with the controls (n =2, 2.5%), χ^2 = 7.4, df = 1, p-value = 0.007.

Biochemical parameters	Control n (%)	Study group n (%)	χ^2 value	df	p-value
IgG anti-cardiolipins	-	14 (17.5)	13.2	1	*0.0003
IgM anti-cardiolipins	2 (2.5)	17 (21.3)	11.7	1	*0.0006
Lupus anti-coagulant	2 (2.5)	13 (16.3)	7.4	1	*0.007

**p-value < 0.05 indicate significance, x^2 = Chi-square statistic.*

Table 6: Prevalence of persistent positivity to anti-phospholipids anti-bodies among participants disaggregated by groups after 12 weeks interval

G. INTERACTIONS BETWEEN PARTICIPANTS' POSITIVITY TO BIOCHEMICAL PARAMETERS

Persistent positivity of participants in the study group to more than one biochemical parameters was estimated. Figure 1: depicts the interactive prevalence of positivity to IgG anti-cardiolipins, IgM anti-cardiolipins and Lupus anti-coagulants after twelve weeks. The prevalence of persistent positivity to IgG anti-cardiolipin and lupus anti-coagulant was 11.3% (n = 9), The prevalence of persistent positivity to IgM anti-cardiolipin and lupus anti-coagulant was 8.8% (n = 7), The prevalence of persistent positivity to IgG anti-cardiolipin and lupus anti-coagulant was 10% (n = 8). Persistent positivity to the three biochemical parameters (IgG anti cardiolipin, and lupus anti-coagulant) occurred in five (6.3%) participants.

In other to further explore the interactions between participants' persistent positivity to bio chemical parameters the interactions between participants' persistent positivity to each of these combinations and persistent positivity to lupus anti-coagulants were explored.

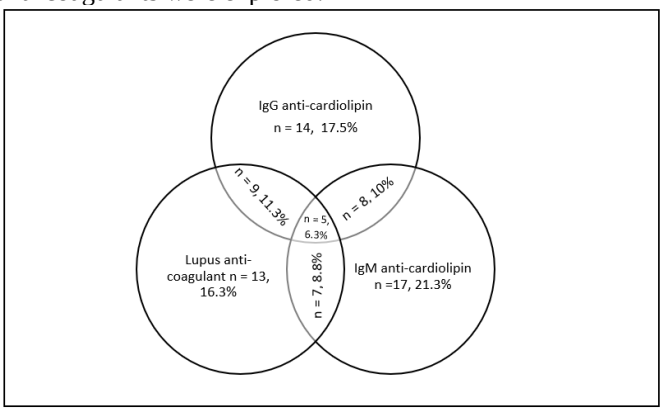

Figure 1: Association between IgG anti-cardiolipin, IgM anti-cardiolipin and Lupus anti-coagulant

Presented in figure 2 below shows the prevalence of persistent positivity to the combinations of the biochemical parameters. Fourteen (17.5%) of participants in the study group were persistently positive to at IgG anti-cardiolipins while 0% of the participants in the control group were positive. Seventeen (21.3%) participants in the study group were persistently positive to at IgM anti-cardiolipins while 2.5% of the participants in the control group were positive. While 13 (16.3%) were persistently positive to lupus anti-coagulant compared to 2.5% in the controls. In general, the overall prevalence of study group participants to anti-phospholipids antibodies was 28.8% and 2.5% in the healthy pregnant women. This implies that 28.8% participants were positive to at least one of the three biochemical parameters.

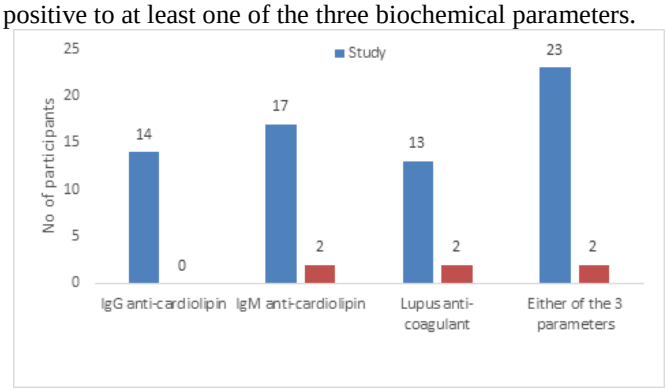

Figure 2: Prevalence of persistent positivity to IgG ACA, IgM ACA and LA

H. RELATIONSHIP BETWEEN PARTICIPANTS STATUS OF ANTI-PHOSPHOLIPIDS ANTIBODIES AND CLINICAL PARAMETERS

Table 7: represents the relationship between persistent positivity to anti-phospholipids antibodies and miscarriages. The total of 14 participants in the study group were positive to IgG anti-cardiolipins of which 3 (21.4%) had experienced at least three miscarriages. Nine (52.9%) of the participants who were positive to IgM anti-cardiolipins had experienced at least 3 miscarriages. The total of 13 participants were found to be positive to Lupus anti-coagulant, of which 23.1% (n=3) had experienced at least 3 miscarriages.

Inductively, there is an association between persistent positivity to IgM anticardiolipins (X^2 = 18.781, p-value <0.001, OR: 13,05, 95% CI for OR: 3.49 – 48.84) and miscarriages (≥ 3).

Ten of the fourteen (71.4%) participants who were positive to IgG anti-cardiolipins were hypertensive. Furthermore, 9 of 17 (52.9%) participants who were positive to IgM anti-cardiolipins had hypertension, and 61.5% (8/13) participants who were positive to lupus anti-coagulant were hypertensive. The persistent positivity among participants to IgG anti-cardiolipins (X^2 = 4.95, p-value = 0.025, OR: 4.67, 95% CI for Odds ratio: 1.32 – 16.65) were associated with hypertension.

Biochemical parameters	Miscarriages n (%) < 3	Miscarriages n (%) ≥ 3	Test statistic	p-value	Odds ratio	95% CI for odds ratio
IgG anti-cardiolipins						
Positive	11 (78.6)	3 (21.4)	X^2 =	0.670	1.36	0.33 –
Negative	55 (83.3)	11 (16.7)	0.181			5.71
IgM anti-cardiolipins						
Positive	8 (47.1)	9 (52.9)	X^2 =	*<0.0	13.05	3.49 –
Negative	58 (92.1)	5 (7.9)	18.781	01		48.84
Lupus anti-coagulant						
Positive	10 (76.9)	3 (23.1)	X^2 =	0.563	1.53	0.36 –
Negative	56 (83.6)	11 (16.4)	0.334			6.47

**p-value < 0.05 indicates significance, df – degree of freedom, χ^2 – Chi square statistic.*

Table 7: Cross tabulation between study group participants' status of anti-phospholipids antibodies against level of miscarriage

I. RELATIONSHIP BETWEEN STUDY GROUP PARTICIPANTS' STATUS OF ANTI-PHOSPHOLIPIDS ANTIBODIES AGAINST PARTICIPANTS HYPERTENSION STATUS

Table 8 indicates that about seventy one percent (71.4%) that is 10 out of 14 participants who were positive to IgG anti-cardiolipins were hypertensive. Furthermore, 9 of 17 (52.9%) participants who were positive to IgM anti-cardiolipins had hypertension, and 61.5% (8/13) participants who were positive to lupus anti-coagulant were hypertensive. The persistent positivity among participants to each of IgG anti-cardiolipins (X^2 = 4.95, p-value = 0.025, OR: 4.67, 95% CI for Odds ratio: 1.32 – 16.65) were associated with hypertension.

Biochemical parameters	Hypertension n (%) Hypertensive	Hypertension n (%) Not hypertensive	Test statistic	p-value	Odds ratio	95% CI for Odds ratio
IgG anti-cardiolipins	10 (71.4)	4 (28.6)	X^2 =	*0.02	4.67	1.32 –
Positive	23 (34.8)	43 (65.2)	4.95	5		16.65
Negative						
IgM anti-cardiolipins	9 (52.9)	8 (47.1)	X^2 =	0.270	1.83	0.62 –
Positive	24 (38.1)	39 (61.9)	1.218			5.38
Negative						
Lupus anti-coagulant	8 (61.5)	5 (38.5)	X^2 =	0.104	2.69	
Positive	25 (37.3)	42 (62.7)	2.636		0.79 – 9.12	
Negative						

**p-value < 0.05 indicates significance, df – degree of freedom, χ^2 – Chi square statistic*

Table 8: Cross tabulation between study group participants' status of anti-phospholipids antibodies against participants hypertension status

IV. DISCUSSION

Earlier studies utilized single antibodies in estimating the prevalence of this potentially preventable disease which resulted in under reporting of the actual prevalence in this part of the world [31], [32].

The mean ages of the study participants was 29.9 ± 4.9 years while the mean age of participants in the control group was 29.7 ± 5.2 years and majority of the participants in both groups aged between 30-34 years. This finding is similar to the age range found in the work done by Nwogoh *et al* at university of Benin Teaching Hospital, Benin City, and by Abdulahi *et.al , both in* Nigeria who obtained the mean age of 31.6 ± 4.7 years and 30.9 ± 5.4 years for the study and control group respectively [17]. Though not significantly lower compared to the study done by Ahamed *et al* who found the mean age to be (32 ± 3.4 years) [6], [20]. Age is an important factor in the likelihood of occurrence of anti-phospholipid syndrome and pregnancy complications. Older age group associated with high risk [18]. Both groups were matched with respect to age and this has nullified the possible effect of age on the occurrence of antiphospholipid syndrome in participants between the groups.

Most of the participants (70.6%) in this study were Christians which reflects the dominance of Christians in Ogbomoso. All the participants in the study group were married while 96.2% in the control group were married. This finding is in tandem with a study by Okeyinka who reported that 78.2% of women in Ogbomoso were married [24].

As expected, the dominant ethnic group of participants (Study group - 93.8%, Control group - 92.5%) was Yoruba since this study was conducted in the South Western part of Nigeria, a Yoruba dominated population. This study revealed that majority of the participants in both groups (57.5%-Control group, 78.8%-study group) had tertiary level of education. This may reflect the fact that more of highly educated people use the hospital being a specialist Centre than non-educated individuals. Many of the participants in the study group (63.8%) and the control group (37.5%) were civil servants. This may be attributable to the preponderance of participants with tertiary level of education in this study.

In this study, there was no statistical significant difference in the mean age at menarche between the study group and the control group. This indifference could be attributed to the concordance in the actual mean age of participants in both groups as well as the fact that menarche has no relationship to the presence of antiphospholipid syndrome. The mean cycle length of menstruation between the study group and the control group do not differ significantly. However, duration of menstrual flow is longer in the study group than the control group, this could be due to probable previous use of substances or medications that could affect their bleeding pattern in the past as well as presence of co-morbidities.

There was a significant difference between the mean gestational age of the participants at first contact between the

study group and control group. This might be due to variable attitude of individuals towards booking.

Majority of the participants in the study group had recurrent foetal wastage with maximum being three in about 14 (17.5%) participants and one or two miscarriages in 36 (45%) participants. History of premature delivery occurred in about 9.2%, and foetal death in 2.5% of the participants. Four (5%) participants in the study group had experienced severe eclampsia. These experiences might not be unconnected to the higher likelihood of occurrence of risk factors such as APS in the study group.

The mean weight of the participants in the study group was significantly higher than that of the control group with mean (70.32 ± 11.5kg vs 65.63 ± 12.51kg). This finding might be due to co-existence of primary antiphospholipid syndrome among obese patients as demonstrated in the study done by Karoline Meyer in Medical University of Graz, Austria [22]. Gary *et al* demonstrated in a retrospective study among overweight and obese patients with venous thromboembolism in Medical University of Graz, Austria found out that primary APS (PAPS) occurs more often in obese patients. It was observed that fibrinogen level increases with weight gain. The elevated inflammatory state in over weight and obese patients might be a reason for the increased APS occurrence [25]. A recent studies have also showed that a small decrease of 10% in the pre-pregnancy weight is associated with 10% lower risk of preeclampsia, gestational DM, preterm delivery, macrosomia and still birth [26], [27].

Thirty-three (41.2%) of the study participants had pregnancy induced hypertension (PIH). Antiphospholipid syndrome has been documented as one of the risk factors for pregnancy induced hypertension and the risk increases with presence of more than one auto-antibodies. According to Yamada *et al*, IgG acl and IgG apE were associated with increased risk of mild PIH while LAC and IgG acl were associated with severe PIH [28].

Early pregnancy is faced with many symptoms, which are associated with physiological, physical and psychological disturbances. This could be because of hormonal changes which affect various organs of the system in the body; the foetus might also be lost; which could lead to frustration without any clue to the cause of the problem. This study adopted the use of IgG or IgM ACA and human lupus anticoagulant using ELISA method.

Persistent positivity to auto-antibodies to phospholipids and phospholipids binding protein syndrome was significantly higher in the study than in the control group. This is not unexpected as the clinical factors such as hypertension and miscarriages, which are the predisposing factors to occurrence of anti-phospholipids syndrome, were evident only in participants in the study group. In this study, the prevalence of APLS among pregnant women with one or more complications such as hypertension, recurrent pregnancy loss was 28.8% and the prevalence of 2.5% among healthy pregnant women.

This finding is higher compared with the study done in Benin City among women with preeclampsia which found the prevalence of 10.0% and 0% among apparently healthy pregnant Nigerian women [17]. The prevalence in this study is also higher compared with the study done in Lagos by Akinbami *et al* where he found the prevalence to be 2.3% using IgG ACA [21]. Awodu *et al* also found a lower prevalence in Benin City (15.4%) using KCT coagulation assay for LA [1]. However, the prevalence of APLS found in this study is consistent with finding done by Ahamed *et a*l among women with unexplained recurrent abortion in Wad Medani Obstetrics and Gynaecological Teaching Hospital, Gezira who found the prevalence to be 26% [20]. The disparity in prevalence between this study and most studies done in the country could be attributed to the fact that this study utilized triple auto-antibodies to detect the occurrence of anti-phospholipids syndrome which is contrary to single auto-antibodies used in other studies. Several retrospective and prospective studies have also shown that triple aPL positivity either from aCL, antiβ2-GP1 and LA positivity correlates more strongly with both thrombosis and pregnancy morbidity than the presence of single or double positivity [29], [30].

In this study, variability occurred in the distribution of IgG and IgM ACA. Twenty participants (25%) were positive for IgG ACA among the study group and 6 of 80 (7.5%) participants in the control group were positive at first contact. Persistent positivity occurred in 14 participants (17.5%) in the study group and none of the participants in the control group after twelve weeks interval. The difference in persistent positivity between the two groups was statistically significant. This finding is consistent with the study done by Thiam *et a*l in Senegal who found the prevalence of IgG ACA to be 21.1% among patients with recurrent spontaneous abortions at the Aristido Le Dautec University Teaching Hospital Centre, Dakar [31]. A lower prevalence was found by Akinbami *et al* in Lagos and Zubaida Garba Abdullahi in Zaria with 2.3% and 7.1% respectively [6], [21]. The difference could be attributable to the fact that smaller sample sizes were considered by these authors compared to this study.

Moreover, twenty-three participants (28.8%) were positive for IgM ACA in the study group and 5 (6.2%) participants in the control group at first contact. There was a statistical significant difference in persistent positivity between the study group (n =17, 21.3%) and the control group (n = 2, 2.5%). This finding agrees with the study by Ahamed *et.al* among women with unexplained recurrent abortion in Gezira state, Sudan who found 11 (22%) out of the study participants to have IgM aCL antibodies [20].

In general, two (2.5%) participants in the control group were persistently positive to at least one of IgG ACA or IGM ACA, and 21 participants (26.3%) were persistently positive to either of IgG ACA or IgM ACA.

Furthermore, fourteen participants (17.5%) were positive for Lupus anticoagulant in the study group while it existence was evident only in 5% of participants in the control group at first contact. There was a statistical significant difference in persistent positivity between the study group (16.3%) and the control group (2.5%) after twelve weeks interval. This was lower than the prevalence 24% obtained by Olaniyi *et al* in Ibadan, Nigeria among pregnant women with recurrent foetal loss (reference). The finding in this study is contrary to other studies by Adelowo and Akinbami who found the prevalence of LA to be 9.3% & 4.35% respectively [21], [23]. Ibrahim et al also found a lower prevalence (4%) in Zaria among pregnant women with recurrent pregnancy losses [34]. This

discrepancy might be due to the variability in methods employed as this study made use of one of the sensitive methods in detecting Lupus anticoagulant.

Many of the participants who were persistently positive to IgG anticardiolipins (78.6%) had fewer than 3 miscarriages and majority were hypertensive (71.4%). Hypertensive participants were 5 times more likely to be positive to IgG ACA, and participants with three or more pregnancy losses were equally likely to be positive as well as participants with fewer numbers of miscarriages. This finding is congruent with the study done in Hokkaido University Graduate School of medicine, Japan by Hideto Yamada *et.al*, who found that participants with pregnancy-induced hypertension were 11.4 times more likely to be positive to IgG ACA [30].

In this study, more than half (52.9%) of participants who were persistently positive to IgM ACA had experienced not fewer than three miscarriages and the same proportion were hypertensive. Persistent positivity was associated with miscarriages (≥3) as participants with 3 or more miscarriages are 13 times more likely to be positive to IgM ACA compared to fewer number of miscarriages, and hypertensive participants are equally likely to be positive compared to non-hypertensive participants. The finding in this study is congruent with the finding in a study carried out among women with recurrent spontaneous miscarriage by Sater *et.al, 2012*, implicating ACA as significantly associated with miscarriages (≥3) [35]. However, it is contrary to the finding by Mirai et.al, 2004, implicating anti-phospholipids not associated with recurrent miscarriage [36]. These differences are reconciled by differences in ethnic background, and sample size. Furthermore, persistent positivity to Lupus anti-coagulant was not associated with number of miscarriages and hypertension.

V. CONCLUSION

This study has shown that the prevalence of antiphospholipid syndrome among pregnant women with previous pregnancy complications such as recurrent foetal wastage, IUGR, premature birth and those with complications such as hypertension in the index pregnancy in LAUTECH Teaching Hospital Ogbomoso is relatively high. It is worthy of note that majority of the participants would have been missed using either single or double antibodies. Therefore, this study has demonstrated that using triple anti-bodies; IgG ACA, IgM ACA and LAC will help in identifying many asymptomatic patients that will benefit from early prophylactic treatment and proper monitoring for good outcome in future pregnancies.

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