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ROLE OF ANTIPHOSPHOLIPID ANTIBODIES IN REPEATED MISCARRIAGES

Original Research

Shazia Awan[1]*, Naheed Parveen[2]
[1]Associate Professor, Gu1 Liaquat University of Medical & Health Sciences, Jamshoro (LUMHS), Pakistan.
[2]Dept. of Gyneacology & Obstetrics, Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan.
Corresponding Author: Shazia Awan, Associate Professor, Gu1 Liaquat University of Medical & Health Sciences, Jamshoro (LUMHS), Pakistan, Shaziaasad38@gmail.com
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ABSTRACT

Background: Recurrent miscarriage (RM), defined as two or more consecutive pregnancy losses before 20 weeks of gestation, is a significant reproductive health concern with multifactorial etiologies. Antiphospholipid antibodies (APAs) are recognized as one of the leading immunological causes of RM, contributing to placental insufficiency and thrombotic events. In Pakistan, limited data exists on the prevalence and clinical significance of APA among women with RM.

Objective: To determine the prevalence and impact of antiphospholipid antibodies in women with recurrent miscarriages attending a tertiary care hospital in Pakistan.

Methods: A case-control observational study was conducted over six months at the Department of Gynecology and Obstetrics, Liaquat University of Medical and Health Sciences, Jamshoro. Sixty-six women were enrolled through consecutive sampling: 33 cases with ≥2 unexplained miscarriages, and 33 controls with at least one successful full-term birth. Sociodemographic data and obstetric history were recorded. Blood samples were tested for lupus anticoagulant (LAC), anticardiolipin antibodies (aCL IgG and IgM), and anti-β2 glycoprotein I antibodies (IgG and IgM). Antibody positivity was confirmed by repeat testing 12 weeks apart. Data were analyzed using SPSS v21.0, with p-value <0.05 considered significant.

Results: APA positivity was significantly higher among cases than controls (LAC: 33.3% vs. 3.0%; aCL IgG: 30.3% vs. 0%; β2-GPI IgG: 27.3% vs. 3.0%; $p < 0.01$ for all). APA-positive cases had more miscarriages (mean: 3.1 vs. 2.4) and earlier gestational losses.

Conclusion: Antiphospholipid antibodies are significantly associated with recurrent miscarriage. Early identification and intervention in APA-positive women may improve pregnancy outcomes. Integration of APA screening into clinical protocols is recommended in similar settings.

Keywords: Anticardiolipin antibodies, Antiphospholipid syndrome, Autoantibodies, β2-glycoprotein I, Lupus anticoagulant, Miscarriage, Pregnancy loss.

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INTRODUCTION

Recurrent pregnancy loss (RPL) is a distressing reproductive challenge affecting a significant number of women worldwide, including a considerable proportion in Pakistan. Defined as the loss of two or more pregnancies before 20 weeks of gestation, RPL has both physical and profound emotional implications for women and their families. Among the multitude of causes associated with RPL, antiphospholipid antibodies (APAs)—a group of autoantibodies that target phospholipid-binding proteins—have emerged as a significant immunological factor. These antibodies are associated with thrombogenic mechanisms that disrupt placental function, leading to fetal loss and other pregnancy-related complications (1). When present in high concentrations and sustained over time, APAs are the hallmark of antiphospholipid syndrome (APS), a disorder known for its capacity to induce vascular thrombosis and pregnancy morbidity (2). APS is diagnosed through a combination of clinical events such as unexplained fetal loss beyond 10 weeks, recurrent early miscarriages, or severe preeclampsia, coupled with the persistent presence of APAs—including lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), and anti-β2 glycoprotein I antibodies—measured on two occasions at least 12 weeks apart (3). These antibodies are believed to contribute to pregnancy loss by interfering with placental circulation, impairing trophoblastic function, and promoting coagulation. Mechanistic studies suggest that APAs reduce the production of prostacyclin by endothelial cells, enhance thromboxane generation by platelets, and inhibit the activation of protein C, culminating in a prothrombotic state (4,5). Furthermore, in vitro studies have shown that heparin can inhibit APA binding in a dose-dependent manner, pointing toward a biological rationale for its therapeutic use (6).

The combination of heparin and low-dose aspirin remains the cornerstone of APA management in pregnancy. Aspirin helps prevent platelet aggregation through its anti-thromboxane effects, while heparin counteracts APA-mediated coagulation and may improve implantation (7,8). Additional therapies such as corticosteroids and intravenous immunoglobulin are thought to exert immunomodulatory effects, reducing autoantibody activity and inflammatory responses (9). Empirical studies report that women with RPL and APAs treated with this therapeutic regimen have a live birth rate of up to 75%, with minimal maternal or fetal complications (10,11). Despite these promising outcomes, awareness and access to APA screening and treatment remain limited in many resource-constrained settings, including Pakistan. Given the cultural and emotional significance of childbearing in Pakistani society and the gaps in local healthcare infrastructure for diagnosing immunological causes of pregnancy loss, it is imperative to explore this condition in greater depth. The psychosocial burden of unexplained miscarriages, compounded by limited diagnostic protocols, highlights an urgent need for locally relevant research. This study, therefore, seeks to investigate the prevalence and clinical impact of antiphospholipid antibodies in Pakistani women who have experienced repeated miscarriages, with the aim of informing future screening guidelines and treatment strategies tailored to the local context.

METHODS

This study employed a case-control observational design conducted at the Department of Gynecology and Obstetrics, Liaquat University of Medical and Health Sciences, Jamshoro. The total duration of the study was six months, commencing after the approval of the research synopsis by the institutional ethical review committee. Ethical principles were strictly adhered to throughout the study, and approval was obtained from the Institutional Review Board. Written informed consent was obtained from all participants after a detailed explanation of the study objectives and procedures. A total of 66 women of reproductive age were enrolled using non-probability, consecutive sampling. The study population was divided into two groups: 33 cases comprised women aged 18–40 years who had experienced at least two unexplained consecutive miscarriages before the 20th week of gestation, while 33 controls included women of the same age group who had experienced at least one full-term live birth with no history of miscarriage. Women were excluded if they had known chromosomal or genetic abnormalities (in either parent or fetus), anatomical uterine defects, or underlying hormonal or metabolic disorders such as thyroid dysfunction or diabetes, to minimize confounding variables (12).

Following enrollment, eligible participants underwent structured interviews using a pre-designed questionnaire to gather detailed sociodemographic information, medical and obstetric history, and miscarriage-related data. Subsequently, 3 mL of venous blood was collected from each participant to test for antiphospholipid antibodies, including lupus anticoagulant (LAC), anticardiolipin antibodies (aCL), and anti-β2 glycoprotein I antibodies. All laboratory analyses were performed using validated immunoassay techniques in a

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standardized setting. Samples that tested positive for any of the antibodies were re-tested after a minimum interval of 12 weeks, as per established diagnostic criteria for antiphospholipid antibody syndrome, to confirm antibody persistence and rule out transient positivity. To ensure participant confidentiality, unique alphanumeric codes were assigned in place of personal identifiers, and the data was stored securely with password protection. All identifiable information will be discarded five years following the completion of the study in compliance with ethical data handling protocols. For statistical analysis, data were entered and managed using Microsoft Excel 2016 and analyzed using SPSS version 21.0. Categorical variables were reported as frequencies and percentages, while continuous variables were summarized as means with standard deviations. Logistic regression analysis was employed to determine the association between antiphospholipid antibodies and repeated miscarriages, adjusting for potential confounding factors. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Out of the total 66 participants enrolled in this study, 33 were cases with a history of two or more consecutive miscarriages, while 33 served as controls with at least one full-term live birth and no history of pregnancy loss. The two groups were similar in terms of mean age and body mass index (BMI), with cases averaging 30.2 ± 4.6 years and a BMI of 26.1 ± 3.2 kg/m², and controls averaging 29.8 ± 4.1 years and a BMI of 25.7 ± 3.4 kg/m². A higher proportion of cases (57.6%) were from rural backgrounds compared to 51.5% of controls, and the majority of both groups fell into the middle socioeconomic class. The average number of pregnancies was higher among cases (3.5) than controls (2.9), while the number of live births was lower in cases (0.7) compared to controls (1.0). Among the case group, the mean number of miscarriages was 2.8, with the average gestational age at the time of miscarriage being 9.4 weeks. In terms of lifestyle factors, smoking was more prevalent in the case group (18.2%) compared to controls (9.1%). Use of medications or supplements during pregnancy was also more common in cases (72.7%) than controls (60.6%). Regarding the antiphospholipid antibody tests, notable differences were observed between groups. In the case group, the lupus anticoagulant (LAC) test was positive in 11 participants (33.3%) on both occasions, whereas only 1 participant (3.0%) in the control group tested positive. Similarly, anticardiolipin IgG antibodies were persistently positive in 10 cases (30.3%) and none of the controls. Anticardiolipin IgM antibodies were positive in 8 cases (24.2%) and remained negative in all controls. For anti-β2 glycoprotein I antibodies, IgG was positive in 9 cases (27.3%) and in 1 control (3.0%), while IgM positivity was observed in 7 cases (21.2%) and none of the controls.

The antibody results were confirmed through repeated testing after a minimum interval of 12 weeks, consistent with established diagnostic protocols. The figures and tables presented demonstrate clear differences in the prevalence of APA positivity between cases and controls, highlighting the significant association between APA presence and recurrent miscarriage. Further statistical analysis revealed significant differences in the prevalence of antiphospholipid antibodies between the case and control groups. Lupus anticoagulant was present in 33.3% of cases versus 3.0% of controls, with a statistically significant p-value of 0.001 and an odds ratio of 15.4 (95% CI: 1.9–123.7). Similarly, anticardiolipin IgG and IgM antibodies showed significantly higher positivity among cases, with controls showing no positivity, yielding undefined odds ratios but highly significant p-values (<0.001). Anti-β2 glycoprotein I antibodies (both IgG and IgM) were also significantly more prevalent in the case group (p-values ranging from 0.002 to 0.004). A stratified subgroup analysis within the case group compared APA-positive versus APA-negative women. It was observed that APA-positive cases had a significantly higher mean number of miscarriages (3.1 vs. 2.4; p = 0.03) and experienced pregnancy losses at a slightly earlier gestational age (mean 8.6 vs. 10.5 weeks; p = 0.04). Smoking prevalence and supplement use were higher among APA-positive cases (22.2% and 77.8%, respectively) compared to APA-negative cases (13.3% and 66.7%), though these differences were not statistically significant. These analyses underscore the clinical relevance of APA testing in women with recurrent pregnancy loss and suggest a potential association between APA positivity and both the frequency and timing of miscarriage events.

Table 1: Demographic Characteristics of Study Participants

Variable	Cases (n=33)	Controls (n=33)
Mean Age (years)	30.2	29.8
Mean BMI (kg/mÂ²)	26.1	25.7
Residence - Rural (%)	57.6	51.5
Residence - Urban (%)	42.4	48.5
Socioeconomic Status - Upper (%)	6.1	9.1

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Variable	Cases (n=33)	Controls (n=33)
Socioeconomic Status - Middle (%)	51.5	57.6
Socioeconomic Status - Lower (%)	42.4	33.3
Mean Number of Pregnancies	3.5	2.9
Mean Number of Live Births	0.7	1
Mean Number of Miscarriages (cases only)	2.8	0
Mean Gestational Age at Miscarriage (weeks)	9.4	0
Smoking Status - Yes (%)	18.2	9.1
Smoking Status - No (%)	81.8	90.9
Use of Medications/Supplements - Yes (%)	72.7	60.6
Use of Medications/Supplements - No (%)	27.3	39.4

Table 2: Lupus Anticoagulant Test Results

Test Phase	Cases (n=33)	Controls (n=33)
First Test - Positive	11	1
First Test - Negative	22	32
Second Test - Positive	11	1
Second Test - Negative	22	32

Table 3: Anticardiolipin Antibody Test Results

Test	Cases (n=33)	Controls (n=33)
IgG First Test - Positive	10	0
IgG First Test - Negative	23	33
IgG Second Test - Positive	10	0
IgG Second Test - Negative	23	33
IgM First Test - Positive	8	0
IgM First Test - Negative	25	33
IgM Second Test - Positive	8	0
IgM Second Test - Negative	25	33

Table 4: Anti-β2 Glycoprotein I Antibody Test Results

Test	Cases (n=33)	Controls (n=33)
IgG First Test - Positive	9	1
IgG First Test - Negative	24	32
IgG Second Test - Positive	9	1
IgG Second Test - Negative	24	32
IgM First Test - Positive	7	0
IgM First Test - Negative	26	33
IgM Second Test - Positive	7	0
IgM Second Test - Negative	26	33

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Table 5: Statistical Comparison Between Groups

APA Marker	Cases Positive (n=33)	Controls Positive (n=33)	P-Value	Odds Ratio (95% CI)
LAC Positive	11	1	0.001	15.4 (1.9-123.7)
aCL IgG Positive	10	0	0.0001	Undefined (high significance)
aCL IgM Positive	8	0	0.0015	Undefined (high significance)
β2-GPI IgG Positive	9	1	0.002	10.1 (1.2-85.3)
β2-GPI IgM Positive	7	0	0.004	Undefined (high significance)

Table 6: Stratified Subgroup Results Within Cases

Variable	APA Positive Cases (n=18)	APA Negative Cases (n=15)	P-Value
Mean Number of Miscarriages	3.1	2.4	0.03
Mean Gestational Age at Miscarriage (weeks)	8.6	10.5	0.04
Smoking Prevalence (%)	22.2	13.3	0.39
Supplement Use (%)	77.8	66.7	0.41

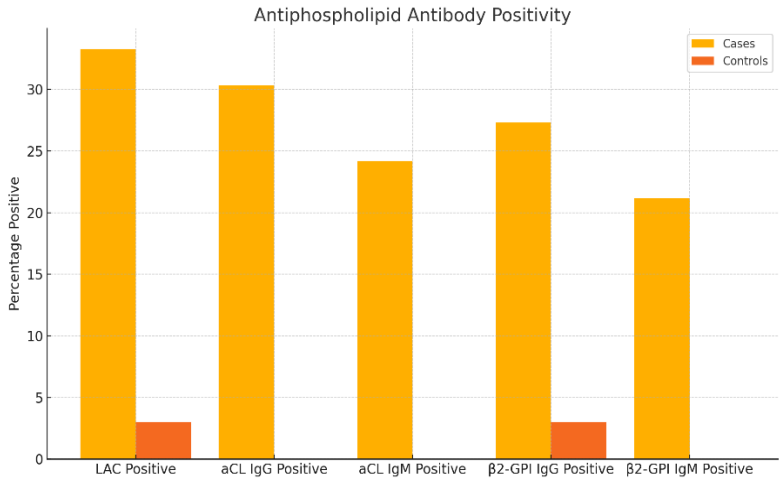

Figure 1 Antiphospholipid Antibody Positivity

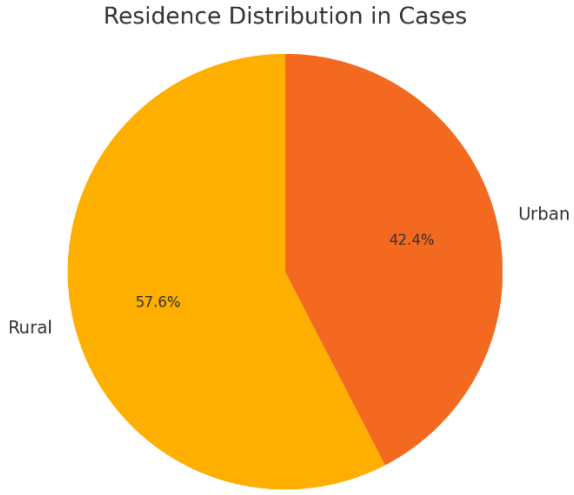

Figure 2 Residence Distribution in Cases

DISCUSSION

The findings of this study highlight a significant association between antiphospholipid antibodies (APAs) and recurrent miscarriage (RM) among Pakistani women of reproductive age. A markedly higher prevalence of lupus anticoagulant, anticardiolipin antibodies, and anti-β2 glycoprotein I antibodies was observed in women with repeated miscarriages compared to those with no miscarriage history. These results reinforce the established understanding that APA positivity is a critical etiological factor in recurrent pregnancy loss, likely due to their interference with placental function and promotion of a hypercoagulable state (13). Consistent with previous research, this study found that APA positivity was significantly more common in cases than in controls, with LAC being the most frequently detected antibody among patients with RM. A study similarly reported elevated anticardiolipin and anti-annexin V antibodies in women with multiple pregnancy losses, with a significant relationship between APA levels and the number of miscarriages (14,15). The data are further supported by findings from studies, reported a 30.5% prevalence of APAs among Sudanese women with RM, indicating the widespread clinical relevance of APA testing in low-resource settings (16-18). Stratified analysis within the case group revealed that

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APA-positive women experienced miscarriages at earlier gestational ages and in greater numbers than their APA-negative counterparts, suggesting that the presence of these antibodies not only increases miscarriage risk but may also influence the timing and recurrence pattern. This is consistent with the findings of a study, which noted a significant association between elevated IgM APA levels and earlier gestational losses in a cohort of Sudanese women (19).

One of the strengths of the present study lies in its use of rigorous diagnostic criteria, including confirmatory APA testing 12 weeks apart, and detailed sociodemographic profiling to control for confounding variables. Additionally, subgroup analysis allowed for more nuanced interpretation of how APA status may differentially affect reproductive outcomes (20,21). However, limitations must be acknowledged. The study's relatively small sample size limits the generalizability of findings, and the non-probability sampling method may have introduced selection bias. Furthermore, the six-month duration may have restricted the ability to retest all positive APA cases, potentially affecting confirmation rates. Another critical limitation is the absence of long-term follow-up on pregnancy outcomes post-treatment among APA-positive participants. Studies have shown that therapeutic interventions, including low-dose aspirin and low-molecular-weight heparin, significantly improve live birth rates in APA-positive women. For instance, a study demonstrated a nearly twofold increase in live births among treated patients with low-titer APAs compared to untreated ones. Including such outcome data in future research would provide valuable insight into the therapeutic implications of APA screening (22). Emerging literature also suggests that non-criteria antiphospholipid antibodies, such as anti-phosphatidylserine/prothrombin, may be relevant in cases where conventional markers are negative. Studies reported significant associations between these non-criteria antibodies and RM, especially in women who test negative for standard APAs. This suggests a potential diagnostic gap that could be addressed by expanding screening protocols (23,24).

The findings underscore the necessity for routine APA screening in women with unexplained pregnancy losses, particularly in regions like Pakistan where awareness and access to immunological investigations remain limited. Integrating APA testing into national maternal health programs could contribute to early diagnosis and personalized management, ultimately reducing miscarriage-related morbidity. Future studies should include larger, multicentric cohorts, long-term pregnancy outcome monitoring, and assessment of therapeutic efficacy in APA-positive women. Incorporating both conventional and non-criteria APAs, alongside markers of inflammation and thrombosis, could also refine the predictive accuracy of diagnostic models.

CONCLUSION

This study demonstrates a significant association between antiphospholipid antibodies and recurrent miscarriage among Pakistani women, underscoring the importance of routine APA screening in cases of unexplained pregnancy loss. The findings support integrating immunological testing into standard obstetric care to improve diagnostic accuracy and guide targeted therapy, ultimately enhancing maternal outcomes and reducing the burden of miscarriage in resource-limited settings.

AUTHOR CONTRIBUTION

Author	Contribution
Shazia Awan*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Naheed Parveen	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published

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