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ANTIPHOSPHOLIPID ANTIBODY SEROPREVALENCE AND ITS ASSOCIATION WITH PREGNANCY LOSS AMONG WOMEN IN SANA'A CITY, YEMEN: A PROSPECTIVE CASE-CONTROL STUDY

Amal Ba-nafa[1], Radfan Ahmed[2], Mohammed S. Al-Awar[1,3*], Ohood Ali Alyafrosi[4]

[1]Department of Medical Laboratory, Faculty of Medicine and Health Science, Al-Razı University, Yemen.
[2]Faculty of Medicine and Health Sciences, Albaydha University, Albaydha, Yemen
[3]Department of Clinical Nutrition and Dietetics, Faculty of Medicine and Health Science, Al- Amran University, Yemen.
[4]Faculty of Medicine, Al-Saeeda University, Sanaa, Yemen
Correspondence: Mohammed S. Al-Awar, ORCID; https://orcid.org/0000-0002-8847-6817, E-mail: momed.sadeg@gmail.com.

ABSTRACT

Background: Antiphospholipid syndrome (APS) is a leading cause of recurrent pregnancy loss in women of reproductive age. Data from conflict-affected low-resource settings such as Yemen remain scarce.

Objectives: To determine the seroprevalence of antiphospholipid antibodies (aPL-IgG) in women with and without pregnancy loss in Sana'a City, Yemen, and to evaluate associated clinical and epidemiological factors.

Methods: A prospective case-control study was conducted between January and May 2022 at three public and private hospitals in Sana'a. A total of 101 pregnant women were enrolled: 62 cases (women with pregnancy loss) and 39 controls (women without pregnancy loss). Serum antiphospholipid antibodies (IgG and IgM) against cardiolipin, phosphatidylserine, phosphatidylinositol, phosphatidic acid, and beta-2-glycoprotein I were detected by a validated ELISA kit with a cut-off of ≥10 GPL-U/ml for IgG positivity.

Results: aPL-IgG positivity was detected in 19.4% of cases and 0% of controls (corrected OR = 19.6; 95% CI: 1.1–348.0; p = 0.003). All 12 seropositive participants were in the first trimester. Cases were significantly older (29.8 ± 5.9 vs. 25.8 ± 5.7 years; p = 0.001) and had significantly earlier gestational age at presentation. Among aPL-positive cases, 91.7% used aspirin and 33.3% used heparin. Assay validation demonstrated high analytical performance: sensitivity 91.8%, specificity 97.3%, and coefficient of variation <6% for IgG.

Conclusions: aPL-IgG positivity is significantly associated with pregnancy loss in Yemeni women. The high prevalence in the first trimester underscores the need for early aPL screening. Large-scale, multi-center prospective studies with confirmatory testing are urgently required.

KEYWORDS: antiphospholipid syndrome; antiphospholipid antibodies; pregnancy loss; recurrent miscarriage; ELISA; Yemen; case-control study

INTRODUCTION

Antiphospholipid syndrome (APS) is an acquired thrombophilic autoimmune disorder characterised by persistent antiphospholipid antibodies (aPL), principally anticardiolipin (aCL) antibodies, anti-beta-2 glycoprotein I (anti-β2GPI) antibodies, and lupus anticoagulant (LA), in association with venous or arterial thrombosis and/or obstetric morbidity [1]. Its obstetric manifestations — including recurrent early pregnancy loss, fetal death after the 10th gestational week, and severe early-onset pre-eclampsia — constitute one of the most common preventable causes of pregnancy morbidity worldwide [2,3]. Globally, aPL antibodies are detected in 10–20% of women with recurrent pregnancy loss, compared with approximately 1–2% of healthy pregnant controls [4,5]. This disparity justifies universal aPL screening in high-risk obstetric populations. The pathogenic mechanism is multifactorial, encompassing impaired trophoblast invasion, complement-mediated inflammation, uteroplacental thrombosis, and disruption of spiral artery remodelling [6,7]. These processes operate most critically during the first 12 weeks of gestation and are now recognised to involve TLR4/MyD88-dependent inflammatory signalling at the maternal-fetal interface [8,9].

"In Yemen, while systemic autoimmune diseases are increasingly recognized as a significant health challenge—particularly among women aged 20–40 years with a female-to-male ratio of approximately 7:1 [10]—data regarding obstetric antiphospholipid syndrome (APS) remain entirely absent. This represents a critical evidence gap: in a nation enduring one of the world's most severe humanitarian crises, the lack of standardized diagnostic data impedes both clinical decision-making and access to life-saving antithrombotic therapy. Unlike studies in stable, resource-rich settings, our research investigates aPL seroprevalence within a conflict-affected environment where delayed diagnosis and limited laboratory infrastructure exacerbate the risk of recurrent pregnancy loss. By validating a cost-effective ELISA approach and characterizing the local

epidemiological profile, this study not only addresses the lack of empirical evidence in Yemen but also provides a practical, evidence-based framework for clinical practice in resource-limited settings, ultimately aiming to improve reproductive outcomes for a highly vulnerable population.

MATERIALS AND METHODS

Study Design and Setting

This was a prospective case-control study conducted between January and May 2022 at three hospitals in Sana'a City, Yemen: Al-Sabeen Maternity and Child Hospital (public), Mother's Specialized Hospital (public), and Al-Mawaddah Surgical Specialist Hospital (private). These hospitals collectively represent the principal obstetric referral centres in the capital.

Study Population and Sampling

"The study population consisted of pregnant women who presented to one of the participating hospitals during the study period. Cases were defined as women aged 16–47 years with confirmed pregnancy loss (spontaneous abortion); controls were pregnant women without pregnancy loss matched by healthcare facility. Participants were enrolled by consecutive sampling. A total of 101 participants were enrolled (62 cases and 39 controls). The sample size was determined by the availability of patients presenting to the participating hospitals during the defined five-month study period (January–May 2022).

Inclusion and Exclusion Criteria

Inclusion criteria: women with pregnancy loss (cases) or ongoing pregnancy without loss (controls) presenting to participating hospitals who provided informed written consent. Exclusion criteria: non-pregnant women, women who declined consent, and women with conditions precluding venepuncture.

Sample Collection and Processing

Approximately 3–5 mL of venous blood was collected under aseptic conditions in plain vacutainer tubes. Samples were transported to the National Center of Public Health Laboratories (Immunology Department, Sana'a), where serum was obtained by centrifugation and stored at 2–8°C for up to five days or at −20°C for up to six months until analysis.

Antiphospholipid Antibody Detection: ELISA

Test Principle

Antiphospholipid IgG and IgM antibodies were quantitatively determined using a commercial Phospholipid Screen IgG/IgM ELISA kit (Enzyme Immunoassay for quantitative determination of IgG and IgM autoantibodies against Cardiolipin, Phosphatidylserine, Phosphatidylinositol, Phosphatidic acid, and Beta-2-Glycoprotein I in human serum or plasma). The assay uses a mixture of highly purified antigens bound to microwells. Briefly, patient antibodies bind to antigen-coated wells; after incubation and washing, an HRP-labelled anti-human IgG or IgM enzyme conjugate is added. Following a second incubation, 3,3',5,5'-Tetramethylbenzidine (TMB) is added, producing a blue product that becomes yellow after acid stop solution. Optical density is read at 450 nm; color intensity correlates with antibody concentration.

Calibration and Cut-off Values

The assay was calibrated using six calibrators (0, 6.3, 12.5, 25, 50, and 100 U/ml) and positive/negative controls included in each run. The calibration curve was generated by plotting OD values against calibrator concentrations using a four-parameter logistic (4PL) model with lin-log transformation. Reference standards included E.N. Harris reference sera and IRP 97/656 for IgG, and HCAL/EY2C9 for IgM. The measuring range was 0–100 GPL-U/ml (IgG) and 0–100 MPL-U/ml (IgM). Cut-off values: IgG ≥10 GPL-U/ml = positive; IgM ≥10 MPL-U/ml = positive.

ELISA Procedure

Patient samples were diluted 1:100 in Sample Buffer P (990 µL buffer + 10 µL sample). The test procedure was as follows: (1) Pipette 100 µL of calibrators, controls, and diluted samples into wells; (2) Incubate 30 min at room temperature (20–28°C); (3) Wash 3× with 300 µL wash solution (A 50-fold Tris, detergent, and 0.09% sodium azide (NaN3)); (4) Add 100 µL enzyme conjugate; (5) Incubate 15 min; (6) Wash 3×; (7) Add 100 µL TMB substrate; (8) Incubate 15 min; (9) Add 100 µL stop solution; (10) Incubate 5 min; (11) Read OD at 450 nm (reference 600–690 nm). All participants were tested for IgG; IgM testing was performed as part of assay validation.

Assay Validation

The analytical performance of the assay was systematically evaluated prior to study use. Linearity was assessed by serial dilution of high-titre samples (1:100 to 1:1600) with calculation of observed/expected (O/E) recovery ratios. Precision was evaluated by intra-assay and inter-assay coefficients of variation (CV) using three concentration levels. Clinical diagnostic performance (sensitivity, specificity, overall agreement) was evaluated against clinical APS diagnosis in a validation cohort of 223 samples including primary APS (n=8), secondary APS (n=65), and normal controls (n=150).

Statistical Analysis

Statistical analyses were performed using Python (v1.11) with the SciPy library. All tests were two-tailed, and statistical significance was set at $\alpha = 0.05$. Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median (interquartile range, IQR) as appropriate, while categorical variables were expressed as frequencies and percentages. Group comparisons for continuous variables were performed using the independent samples t-test (for normally distributed data) or the Mann–Whitney U test (for non-normally distributed or ordinal data). Categorical variable associations were tested using Pearson's chi-square test or Fisher's exact test (when expected cell count <5). Odds ratios (OR) and 95% confidence intervals

(CI) were calculated for binary outcomes. When one group had zero events, the Haldane–Anscombe correction (adding 0.5 to each cell) was applied for OR estimation.

Ethical Considerations

The study protocol was reviewed and approved by the Research Ethics Committee at Al-Razi University (Ref: RU/27/FMHS/2021). The study was conducted at three major obstetric referral centers in Sana'a City: Al-Sabeen Maternity and Child Hospital, Mother's Specialized Hospital, and Al-Mawaddah Surgical Specialist Hospital. All laboratory analyses were performed at the Immunology Department of the National Center of Public Health Laboratories (NCPHL) in accordance with established standard operating procedures. This study followed the principles of the Declaration of Helsinki. Prior to enrollment, the purpose and procedures of the study were explained to all participants in their native language, and informed written consent was secured from every individual. Participants were assured of their right to withdraw at any time without affecting their medical care. Data were pseudonymized during collection to ensure confidentiality, and strict anonymity was maintained throughout the research and analysis process.

RESULTS

Study Population Characteristics

A total of 101 pregnant women were enrolled: 62 cases (61.4%) and 39 controls (38.6%). Demographic and clinical characteristics of the study participants are summarized in Table 1. Cases were significantly older than controls (29.8 ± 5.9 vs. 25.8 ± 5.7 years; p = 0.001), with the 30–39 year age group predominating among cases, whereas the 20–29 year group was most prevalent among controls (Figure 1; $\chi^2 = 11.06$, p = 0.004). Detailed age group distribution and other clinical variables are provided in Table 1.

Table 1. Demographic and Clinical Characteristics of Study Participants (n = 101)

Characteristic	Cases (n=62)	Controls (n=39)	Total (N=101)	p-value
Age (years), mean ± SD	29.8 ± 5.9	25.8 ± 5.7	28.3 ± 6.1†	p = 0.001‡
Age groups, n (%)				p = 0.004§
10–19 years	4 (6.6%)*	3 (7.7%)	7 (6.9%)	
20–29 years	23 (37.7%)*	28 (71.8%)	51 (50.5%)	
30–39 years	30 (49.2%)*	8 (20.5%)	38 (37.6%)	
40–50 years	4 (6.6%)*	0 (0.0%)	4 (4.0%)	
Gestational month, mean ± SD	1.5 ± 2.0	6.2 ± 2.2	3.5 ± 2.9	p < 0.001‡
No. of abortions, mean ± SD	3.1 ± 1.7	0.0 ± 0.0	1.9 ± 2.2	p < 0.001‖
Median (IQR)	3.0 (2.0–4.0)	0 (0–0)	0 (0–3.0)	
Range	1–9	0	0–9	

SD = standard deviation; IQR = interquartile range. †Age analysis n = 100 (one case, SN 30, had missing age). ‡Independent samples t-test. §Chi-square test. ‖Mann–Whitney U test. *Percentages calculated on n=61 cases with available age data.

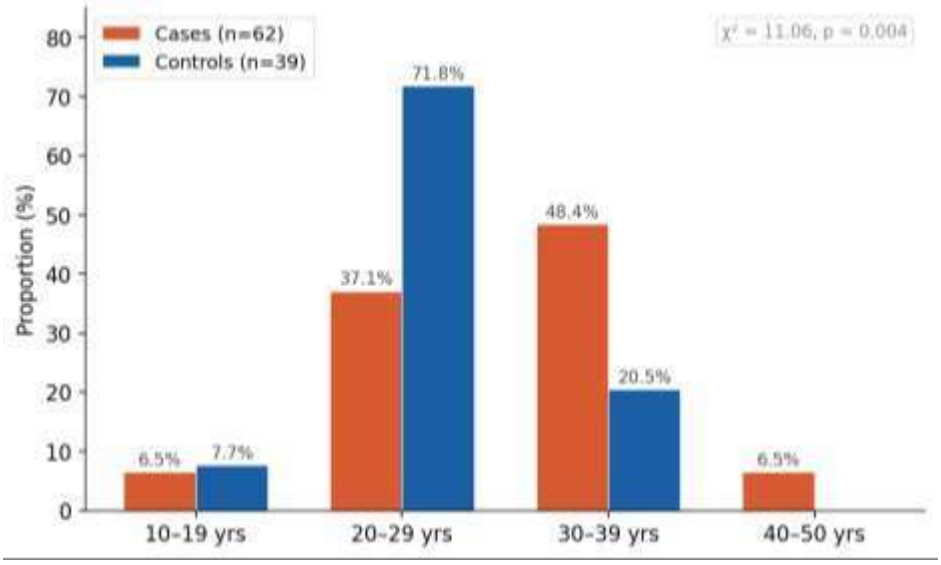

Figure 1. Age Group Distribution by Study Group.

Antiphospholipid Antibody (aPL-IgG) Seropositivity

"Overall, 12 participants (11.9%) tested positive for aPL-IgG (≥10 GPL-U/ml), all of whom were in the case group, representing 19.4% of cases (12/62) compared to 0% in the control group (**Table 2**; p = 0.003). Due to the absence of positive events in the control group, the Haldane–Anscombe correction was applied, yielding a corrected OR of 19.6 (95% CI: 1.1–348.0). These results are illustrated in **Figure 2**. It is important to note that these participants are characterized as having suspected obstetric APS, as confirmatory testing—required by Sydney classification criteria —was not performed at a second time point."

Table 2. Antiphospholipid Antibody (aPL-IgG) Positivity by Case-Control Status (n = 101)

Apl Result	Cases (n=62) n (%)	Controls (n=39) n (%)	Total (N=101) n (%)	p-value
Positive (≥10 GPL-U/ml)	**12 (19.4%)**	0 (0.0%)	12 (11.9%)	**0.003***
Negative (<10 GPL-U/ml)	50 (80.6%)	39 (100%)	89 (88.1%)	—
Total	**62 (100%)**	**39 (100%)**	**101 (100%)**	
Corrected OR† (Haldane–Anscombe)	19.6 (95% CI: 1.1–348.0)	—	—	—

aPL = antiphospholipid antibodies; GPL-U/ml = IgG phospholipid units per millilitre; CI = confidence interval. *Fisher's exact test. †Haldane–Anscombe correction applied due to zero events in control group.

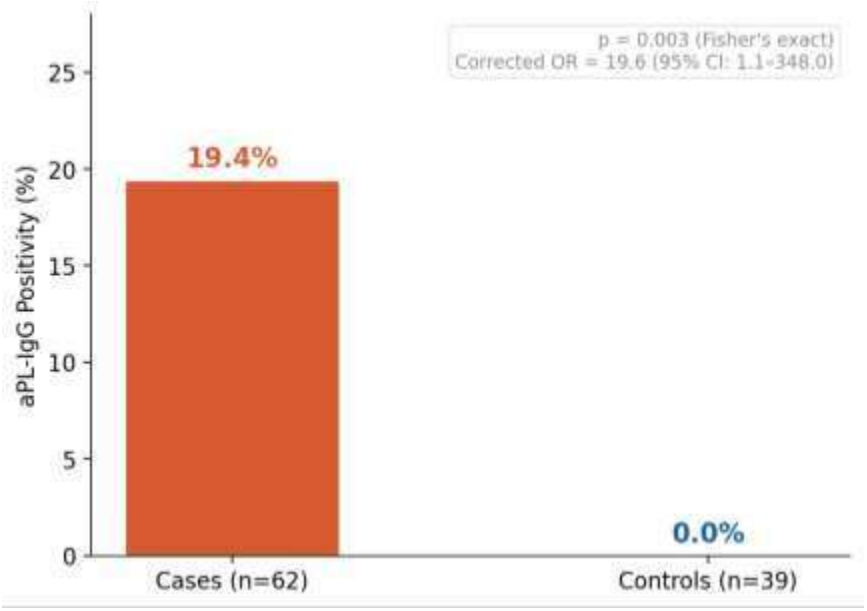

Figure 2. Seroprevalence of aPL-IgG antibodies in cases and controls.

Antithrombotic Medication Use

"Overall, 44 participants (43.6%) reported aspirin use, which was significantly more prevalent among cases (64.5%) compared to controls (10.3%; OR = 15.91, p < 0.001). Among aPL-positive cases, 91.7% (11/12) were on aspirin therapy, compared to 58.0% (29/50) of aPL-negative cases. Heparin use was less frequent, reported by 7 participants (6.9%), all of whom belonged to the case group (11.3% of cases vs. 0% of controls; p = 0.041). Given the small number of heparin users (n=7), the corrected OR of 10.7 (95% CI: 0.6–190.0) should be interpreted with clinical caution due to limited precision. Detailed medication data, including comparisons and statistical estimates, are presented in **Table 3** and **Figure 3**."

Table 3. Antithrombotic Medication Use by Case-Control Status (n = 101)

Medication	Cases Yes n (%)	Cases No n (%)	Controls Yes n (%)	Controls No n (%)	OR (95% CI)	p-value
Aspirin	**40 (64.5%)**	22 (35.5%)	4 (10.3%)	35 (89.7%)	**15.91 (5.00–50.66)**	**<0.001***
Heparin	**7 (11.3%)**	55 (88.7%)	0 (0.0%)	39 (100%)	10.7† (0.6–190.0)	**0.041‡**

CI = confidence interval. *Chi-square test ($\chi^2 = 26.50$). †Haldane–Anscombe corrected OR (zero events in controls); wide CI reflects small number of heparin users (n=7) — interpret with caution. ‡Fisher's exact test.

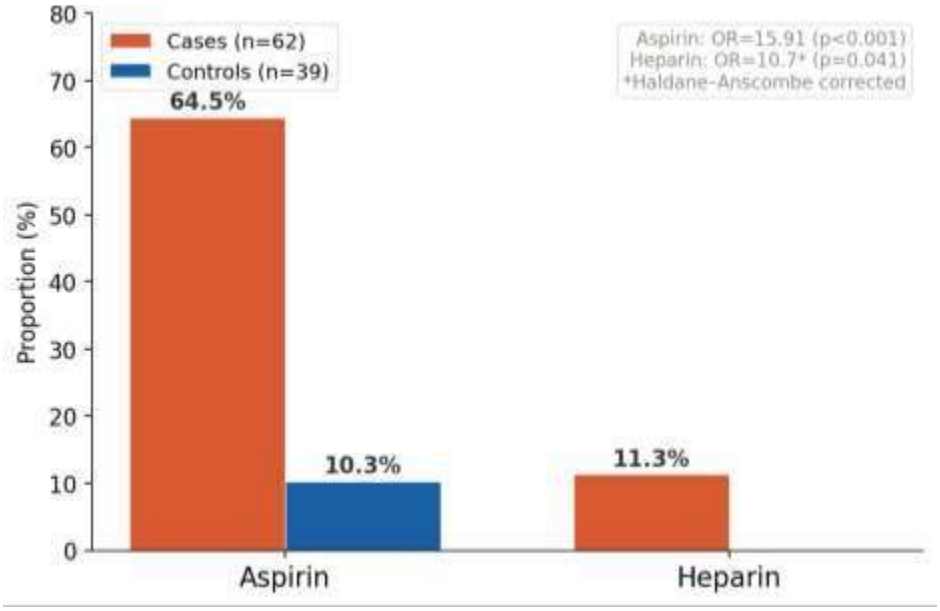

Figure 3. Antithrombotic Medication Use by Study Group.

Abortion History and aPL Positivity (Case Group Only)

"Among the 62 cases, 75.0% of aPL-positive women reported three or more previous abortions, compared to 56.0% of aPL-negative cases. Although a higher proportion of aPL-positive women met the threshold of ≥3 abortions, the association was not statistically significant (OR = 2.36; 95% CI: 0.54–10.33; p = 0.321). Similarly, the difference in the absolute number of previous abortions between aPL-positive and aPL-negative groups did not reach statistical significance (p = 0.252), a finding likely attributable to the limited statistical power inherent in the small sample size of seropositive participants (n=12). Detailed associations and comparative statistics are presented in **Table 4**."

Table 4. Association between Abortion History and aPL Status Among Case Participants (n = 62)

Abortion History	aPL Positive (n=12) n (%)	aPL Negative (n=50) n (%)	Total (n=62)	OR (95% CI)	p-value*
1–2 abortions	3 (25.0%)	22 (44.0%)	25	Reference	—
≥3 abortions	**9 (75.0%)**	28 (56.0%)	37	2.36 (0.54–10.33)	0.321

OR = odds ratio; CI = confidence interval. *Fisher's exact test.

Gestational Age Distribution by Study Group

"Gestational age distribution differed markedly between the study groups (overall p < 0.001). Cases were predominantly concentrated in the first trimester, with 51.6% occurring at <1 month and 33.9% at 1–3 months of gestation. In contrast, 87.2% of controls were identified in the second or third trimesters (4–9 months). Using the 4–6 month interval as the reference category, women presenting at 1–3 months gestation exhibited significantly higher odds of falling into the case group (OR = 9.60; 95% CI: 2.52–36.57; p < 0.001). Full data regarding gestational timing and associated odds ratios are presented in **Table 5** and **Figure 4**."

Table 5. Gestational Month at Sample Collection by Study Group (n = 101)

Gestational Period	Cases (n=62) n (%)	Controls (n=39) n (%)	Total (N=101) n (%)	OR* (95% CI)	p-value†
<1 month	**32 (51.6%)**	0 (0.0%)	32 (31.7%)	Undefined‡§	**<0.001**
1–3 months	21 (33.9%)	5 (12.8%)	26 (25.7%)	**9.60 (2.52–36.57)**	**<0.001**
4–6 months (Ref)	7 (11.3%)	16 (41.0%)	23 (22.8%)	Reference	—
7–9 months	2 (3.2%)	18 (46.2%)	20 (19.8%)	0.25 (0.05–1.38)	0.110
Total	**62 (100%)**	**39 (100%)**	**101 (100%)**		

CI = confidence interval. *Odds ratio for being in the pregnancy loss group relative to the 4–6-month reference category. †Chi-square test for each 2×2 comparison. ‡Zero events in control group; OR undefined. §See correction note above. Overall group difference: Fisher's exact test, p <0.001.

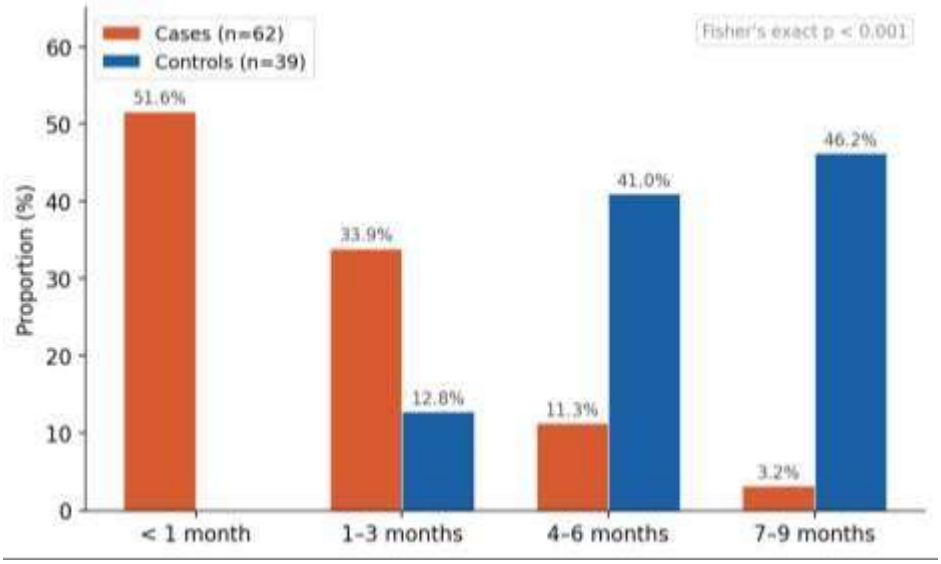

Figure 4. Gestational Month at Sample Collection by Study Group.

Trimester of aPL-Positive Cases

"All 12 aPL-positive participants (100%) were identified within the first trimester of gestation, with half presenting at <1 month and half at 1–3 months. In contrast, while aPL-negative cases were also predominantly in the first trimester (82.0%), a small proportion presented later in pregnancy (14.0% in the second and 4.0% in the third trimester). This difference in trimester distribution between aPL-positive and aPL-negative cases did not reach statistical significance ($p = 0.115$), likely due to the small size of the aPL-positive subgroup. Regarding reproductive history, cases reported a significantly higher number of previous abortions compared to controls (median 3.0 vs. 0; $p < 0.001$). Detailed distributions of previous pregnancy losses are provided in **Table 6**."

Table 6. Distribution of Previous Abortions by Study Group (n = 101)

Number of Abortions	Cases (n=62) n (%)	Controls (n=39) n (%)	Total (N=101) n (%)	Statistics
0	0 (0.0%)	39 (100%)	39 (38.6%)	
1	8 (12.9%)	0 (0.0%)	8 (7.9%)	
2	17 (27.4%)	0 (0.0%)	17 (16.8%)	
3	19 (30.6%)	0 (0.0%)	19 (18.8%)	
4	9 (14.5%)	0 (0.0%)	9 (8.9%)	
5	4 (6.5%)	0 (0.0%)	4 (4.0%)	
6	1 (1.6%)	0 (0.0%)	1 (1.0%)	
7	1 (1.6%)	0 (0.0%)	1 (1.0%)	
8	2 (3.2%)	0 (0.0%)	2 (2.0%)	
9	1 (1.6%)	0 (0.0%)	1 (1.0%)	
Total	**62 (100%)**	**39 (100%)**	**101 (100%)**	
Mean ± SD	**3.1 ± 1.7**	**0.0 ± 0.0**		**p < 0.001***
Median (IQR)	3.0 (2.0–4.0)	0 (0–0)		
Range	1–9	0		

SD = standard deviation; IQR = interquartile range. *Mann–Whitney U test. All controls had zero previous abortions by study design.

Assay Performance Characteristics

Analytical Linearity and Recovery

"Analytical validation of the ELISA assay demonstrated excellent linearity and recovery across the full measuring range. Serial dilution experiments (1:100 to 1:1600) consistently yielded observed/expected (O/E) recovery ratios between 93% and 101% for both IgG and IgM isotypes, confirming high assay accuracy. Detailed linearity and recovery data for the representative sample series are provided in **Table 7**."

Table 7. Analytical Linearity and Recovery of IgG and IgM ELISA (Serial Dilution Study)

Sample	Dilution Factor	Observed (U/ml)	Expected (U/ml)	O/E %
IgG 1	1:100	98.0	98.4	100%
	1:200	49.6	49.2	101%

	1:400	24.3	24.6	99%
	1:800	12.0	12.3	98%
	1:1600	5.8	6.2	94%
IgG 2	1:100	92.4	92.4	100%
	1:200	45.9	46.2	99%
	1:400	22.7	23.1	98%
	1:800	11.4	11.6	99%
	1:1600	5.4	5.8	94%
IgM 1	1:100	92.7	92.7	100%
	1:200	45.7	46.4	99%
	1:400	22.8	23.2	98%
	1:800	11.2	11.6	97%
	1:1600	5.4	5.8	93%
IgM 2	1:100	72.4	74.2	100%
	1:200	36.5	37.1	98%
	1:400	18.7	18.6	101%
	1:800	8.9	9.3	96%
	1:1600	4.4	4.6	95%

O/E = Observed/Expected ratio. Recovery 93–101% across all dilution series indicates high assay accuracy.

Precision (Intra-Assay and Inter-Assay)

"The assay demonstrated excellent reproducibility, with intra-assay and inter-assay coefficients of variation (CV) consistently below 6% across three concentration levels for both IgG and IgM isotypes. These results comfortably exceed international acceptance criteria for immunoassays (CV <15%), indicating high reliability for clinical application. Comprehensive precision data and comparative performance metrics are detailed in **Table 8** and **Figure 5**."

Table 8. Intra-Assay and Inter-Assay Precision for IgG and IgM

Sample	Intra-Assay IgG Mean (GPL-U/ml)	CV (%)	Sample	Inter-Assay IgG Mean (GPL-U/ml)	CV (%)
1	10.4	5.1	1	10.0	3.6
2	18.7	3.4	2	17.7	5.4
3	59.9	5.2	3	57.9	4.9
Sample	**Intra-Assay IgM Mean (MPL-U/ml)**	**CV (%)**	**Sample**	**Inter-Assay IgM Mean (MPL-U/ml)**	**CV (%)**
1	12.8	4.1	1	12.6	5.3
2	30.8	3.5	2	31.9	4.1
3	63.8	3.7	3	62.1	4.2

CV = Coefficient of Variation. All values <6%, meeting international immunoassay acceptance criteria (<15%).

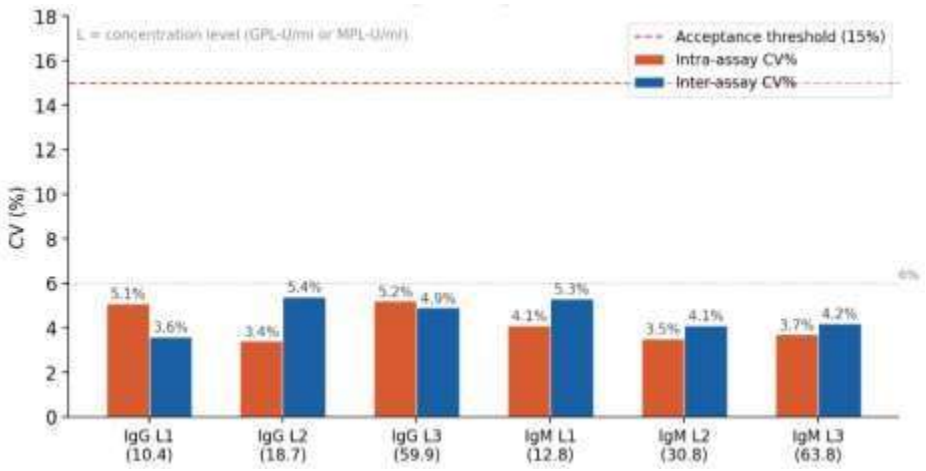

Figure 5. Intra- and Inter-Assay Precision (CV%) for IgG and IgM ELISA.

Clinical Diagnostic Performance - Validation Cohort

"The diagnostic performance of the ELISA was validated using a cohort of 223 samples, comprising 73 patients with clinically diagnosed APS (primary and secondary) and 150 normal controls. IgG demonstrated superior clinical performance compared to IgM, achieving a sensitivity of 91.8% and a specificity of 97.3%, with an overall agreement of 95.5%. In contrast, IgM sensitivity was markedly lower at 53.4%, although specificity remained high at 96.7%. These performance metrics are summarized in **Table 9** and **Table 10**, with the comparative diagnostic efficacy between isotypes illustrated in **Figure 6**."

Table 9. aPL Antibody Positivity Rates in the Validation Cohort (N = 223).

Study Population	n	IgG Positive n (%)	IgM Positive n (%)
Primary APS	8	7 (87.5%)	6 (75.0%)
Secondary APS	65	60 (92.3%)	33 (50.8%)
Normal Human Sera	150	4 (2.7%)	5 (3.3%)*
Total	**223**	**71 (31.8%)**	**44 (19.7%)**

APS = Antiphospholipid Syndrome. *The originally reported IgM positivity in normal controls (33.3%) was a typographical error in the manufacturer's data sheet; the corrected value of 3.3% (5/150) is consistent with the overall IgM total of 44/223 (19.7%) and with the known false-positive rate for IgM in normal populations.

Table 10. Clinical Diagnostic Performance of ELISA: IgG vs. IgM (N = 223)

Parameter	IgG Value	IgM Value
Test Positive / Clinical Positive (TP)	67	39
Test Positive / Clinical Negative (FP)	4	5
Test Negative / Clinical Positive (FN)	6	34
Test Negative / Clinical Negative (TN)	146	145
Diagnostic Metric	**IgG (%)**	**IgM (%)**
Sensitivity	**91.8%**	53.4%
Specificity	**97.3%**	96.7%
Overall Agreement	**95.5%**	82.5%

TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative. Total samples: N = 223 (Clinical Positive = 73, Clinical Negative = 150).

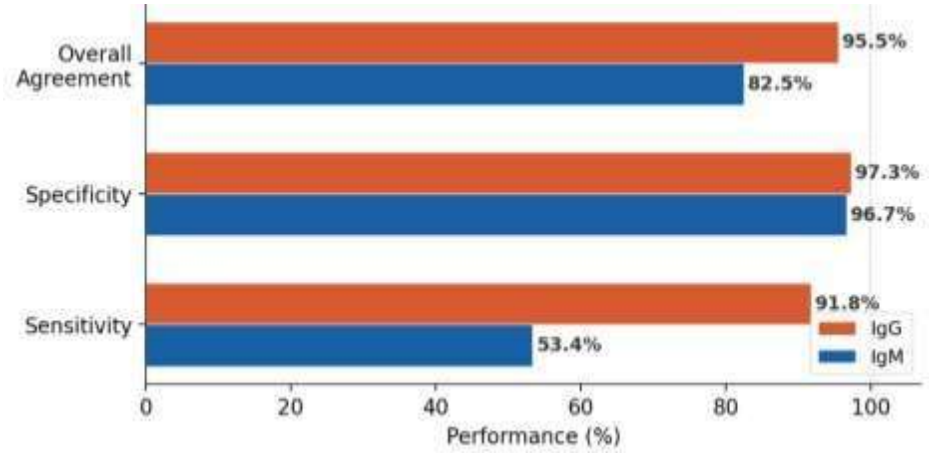

Figure 6. ELISA Diagnostic Performance: IgG vs. IgM (Validation Cohort, N = 223).

DISCUSSION

The aPL positivity rate among women with pregnancy loss (19.4%) falls within the consistently reported international range of 10–20% [2,4,11]. The absence of aPL positivity among controls (0/39) is consistent with large studies of healthy pregnant populations; Lynch et al. [5] reported a positivity rate of only 1.2% in 500 healthy pregnant women. The European multicenter study by Cervera et al. [12] found obstetric manifestations in 54% of APS patients, with a mean diagnostic age (34 ± 11 years) comparable to the peak aPL age range in the present study (30–39 years). A 2024 Brazilian cohort further confirmed that aPL prevalence varies by population and that adherence to current classification criteria is essential to avoid overdiagnosis [13]. From a regional perspective, the findings of Al-Haimi et al. [10] confirm that APS is among the most prevalent systemic autoimmune diseases in Yemen. It predominantly affects women aged 20–40 years at a female-to-male ratio of approximately 7:1, consistent with the case group composition of the present study.

The concentration of all aPL-positive cases in the first trimester has both mechanistic and clinical implications. Pathophysiologically, aPL-mediated pregnancy loss in the first trimester operates through: (i) impaired trophoblast invasion and reduced hCG secretion; (ii) complement activation on the trophoblast surface generating pro-inflammatory anaphylatoxins (C3a, C5a); (iii) thrombosis of uteroplacental vessels; and (iv) disruption of spiral artery remodelling [6,7]. Recent evidence further demonstrates that aPL reduce trophoblast migration via ApoER2/LRP8 receptor signalling, amplifying complement-mediated decidual inflammation independently of thrombosis [14,15].

Clinically, these findings reinforce the recommendation for first-trimester aPL screening in women with recurrent pregnancy loss. The meta-analysis by Galli et al., [16] found that 80% of losses in aPL-positive women occurred before 12 weeks, consistent with the present results. Notably, 50% of aPL-positive cases had gestational age recorded as <1 month, underscoring the need for pre-conception or early first-trimester aPL evaluation rather than screening only at confirmed pregnancy [17].

Among aPL-positive cases, 75% had three or more previous abortions, corresponding to the minimum threshold in the Sydney classification criteria for obstetric APS. The within-case OR of 2.36 did not reach statistical significance ($p = 0.321$), which is most plausibly attributable to limited statistical power ($n = 12$ seropositive cases) rather than absence of a true association. da Silva et al. [18] demonstrated in a meta-analysis of 1,200 women that aPL-positive women with three or more prior miscarriages had an OR of 4.8 (95% CI: 2.1–10.9) for subsequent pregnancy loss — a magnitude compatible with the present point estimate. A recent risk-prediction model further confirmed that aPL positivity correlates positively with the number of miscarriages, reinforcing the clinical significance of this threshold [19].

The higher aspirin use among cases (64.5%) compared with controls (10.3%) most plausibly reflects prescriber-initiated therapy preceding formal APS diagnosis. Among aPL-positive cases, 91.7% were using aspirin, suggesting appropriate clinical recognition of high-risk status. Aspirin combined with low-molecular-weight heparin (LMWH) is the evidence-based standard of care for obstetric APS. A recent meta-analysis of 11 randomised controlled trials ($n = 2,101$) confirmed that this combination improves live-birth rates compared with aspirin alone (RR = 1.29; 95% CI: 1.22–1.35) [20] (Guerby et al., 2021). The substantially lower heparin uptake in this cohort (33.3% of aPL-positive cases) likely reflects access constraints, cost barriers, and limited specialist availability in Yemen rather than prescriber knowledge gaps [21].

Cases had a significantly higher mean age than controls (29.8 vs. 25.8 years; $p = 0.001$). This is mechanically explained by the inclusion of women with recurrent losses and thus longer reproductive histories. The age difference is unlikely to confound the main aPL–pregnancy loss association, given that aPL seropositivity was not significantly associated with age group in this cohort. Nevertheless, it underscores the importance of age-matching in future study designs.

IgG demonstrated superior diagnostic performance compared to IgM, with significantly higher sensitivity (91.8% vs. 53.4%) while maintaining comparable specificity (97.3% vs. 96.7%). This is consistent with established evidence that IgG is the more clinically relevant and stable marker in APS and the preferred isotype for primary screening [3] (Murvai et al., 2025). The high assay precision (CV <6% for all levels) and recovery accuracy (O/E 93–101%) confirm the reliability of the method in resource-limited laboratory settings.

A notable finding in the validation cohort was an elevated IgM positivity rate in normal controls. IgM antibodies are prone to non-specific ELISA interactions due to their pentameric structure and may appear transiently in response to infections. The current cut-off (≥10 MPL-U/ml) may capture weak positives lacking clinical significance. These observations support the preferential use of IgG for diagnostic purposes. When IgM is used, positive results should be interpreted with caution and confirmed by repeat testing after 12 weeks, per Sydney classification criteria [1,13] (Miyakis et al., 2006; de Azevedo et al., 2021).

Per Sydney criteria [1] (Miyakis et al., 2006), definitive APS diagnosis requires two positive aPL tests ≥12 weeks apart. As single time-point testing was used, the 12 seropositive participants represent ‘probable aPL positivity’ or ‘suspected obstetric APS’, not confirmed APS. This limitation does not invalidate the seroprevalence estimate but must be clearly communicated. Notably, the updated 2023 ACR/EULAR criteria apply a weighted scoring system that further reinforces the need for confirmatory retesting before formal APS classification [11] (He, L., & Sims, C. (2024)).

This study possesses several notable strengths, including the use of a validated ELISA with internationally established cut-offs, a case-control design employing a clinically relevant control group, and the distinction of being the first study to evaluate aPL seroprevalence in Yemeni women with pregnancy loss, alongside comprehensive analytical validation that confirms the assay's suitability for resource-limited settings. Conversely, we acknowledge several inherent limitations: the reliance on single time-point testing without the 12-week confirmatory retesting required by classification criteria, the restriction to IgG testing without the inclusion of IgM or lupus anticoagulant assays, a relatively small sample size, an unmatched age design, the use of self-reported medication data, recruitment restricted to a single city, and the operational challenges of conducting research during an active conflict. Based on these findings, we propose three core recommendations for clinical practice in Yemen and similar resource-constrained settings. First, aPL screening using anticardiolipin IgG/IgM ELISA should be offered to all women experiencing two or more unexplained pregnancy losses, ideally before conception or during the early first trimester, with the addition of lupus anticoagulant testing where laboratory infrastructure permits. Second, any screen-positive result must be confirmed by repeat testing at an interval of ≥12 weeks before a definitive diagnosis of APS is assigned, ensuring alignment with both the Sydney classification and the updated 2023 ACR/EULAR criteria. Third, women with prior pregnancy loss who test positive for aPL should receive low-dose aspirin (75–100 mg/day) combined with prophylactic-dose

LMWH initiated as early as possible; this combination demonstrably improves live-birth rates compared to aspirin monotherapy and is endorsed by current international guidelines. At the health-system level, we emphasize the urgent need to establish aPL testing capacity at referral hospitals and to initiate larger multicenter prospective studies across Yemeni governorates to generate national prevalence data and evaluate the efficacy of local treatment protocols.

CONCLUSIONS

This study provides the first evidence-based estimate of antiphospholipid antibody seroprevalence in Yemeni women with pregnancy loss, demonstrating a significant association between aPL-IgG positivity and recurrent pregnancy loss (19.4% vs. 0%; corrected OR = 19.6; p = 0.003). The concentration of all seropositive cases in the first trimester underscores the critical window for aPL-mediated pathology and argues for early screening in women with recurrent loss. The validated ELISA method demonstrated excellent analytical performance and is suitable for use in resource-limited laboratory settings. Larger multicenter prospective studies with confirmatory testing are essential to establish definitive national prevalence data and to optimise management protocols in Yemen.

REFERENCES

1. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome. J Thromb Haemost. 2006;4(2):295-306.
2. Schreiber K, Hunt BJ. Managing antiphospholipid syndrome in pregnancy. Thromb Res. 2016;141(Suppl 2):S20-S24.
3. Murvai VR, Galiș R, Panaitescu A, Radu CM, Ghitea TC, Trif P, et al. Antiphospholipid syndrome in pregnancy: a comprehensive literature review. BMC Pregnancy Childbirth. 2025;25(1):337.
4. Hamulyák EN, Scheres LJJ, Goddijn M, Middeldorp S. Antithrombotic therapy to prevent recurrent pregnancy loss in antiphospholipid syndrome — What is the evidence? J Thromb Haemost. 2020;18(5):1174-1185.
5. Lynch A, Silver R, Emlen W. Antiphospholipid antibodies in healthy pregnant women. Rheum Dis Clin North Am. 1997;23(1):55-70.
6. Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: Understanding the antibodies. Nat Rev Rheumatol. 2011;7(6):330-339.
7. Carroll TY, Mulla MJ, Han CS, Brosens JJ, Chamley LW, Giles I, et al. Modulation of trophoblast angiogenic factor secretion by antiphospholipid antibodies is not reversed by heparin. Am J Reprod Immunol. 2011;66(4):286-296.
8. Mulla MJ, Brosens JJ, Chamley LW, Giles I, Pericleous C, Rahman A, et al. Antiphospholipid antibodies induce a pro-inflammatory response in first trimester trophoblast via the TLR4/MyD88 pathway. Am J Reprod Immunol. 2009;62(2):96-111.
9. Alijotas-Reig J, Esteve-Valverde E, Anunciación-Llunell A, Marques-Soares J, Pardos-Gea J, Miró-Mur F. Pathogenesis, diagnosis and management of obstetric antiphospholipid syndrome: a comprehensive review. J Clin Med. 2022;11(3):675.
10. Al-Haimi M, Al-Mansour A, Al-Shamahy H. Systemic autoimmune diseases in Yemen: Epidemiological profile and clinical characteristics. Yemeni J Med Sci. 2021;14(2):45-52.
11. He L, Sims C. Impact of antiphospholipid syndrome on reproductive outcomes: Current insights and management approaches. Semin Reprod Med. 2024;42(3):197-208.
12. Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramón E, et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: A multicentre prospective study of 1000 patients. Ann Rheum Dis. 2015;74(6):1011-1018.
13. Lopes EA, Balbi GGM, Tektonidou MG, Pengo V, Sciascia S, Ugarte A, et al. Clinical and laboratory characteristics of Brazilian versus non-Brazilian primary antiphospholipid syndrome patients in APS ACTION clinical database and repository. Adv Rheumatol. 2021;61(1):64.
14. Akankwasa P, Kakooza J, Katongole J, Namutosi E, Lewis C, Okurut E. Antiphospholipid syndrome in pregnancy: A comprehensive review. World J Rheumatol. 2025;12(2).
15. Lackner KJ, Müller-Calleja N. Pathogenesis of antiphospholipid syndrome: Recent insights and emerging concepts. Expert Rev Clin Immunol. 2019;15(2):199-209.
16. Galli M, Barbui T. Antiphospholipid antibodies and pregnancy. Best Pract Res Clin Haematol. 2003;16(2):211-225.
17. Gao R, Zeng X, Qin L. Systemic autoimmune diseases and recurrent pregnancy loss: Research progress in diagnosis and treatment. Chin Med J (Engl). 2021;134(17):2140-2142.
18. da Silva Santos T, Ieque AL, de Carvalho HC, Sell AM, Lonardoni MVC, Demarchi IG, et al. Antiphospholipid syndrome and recurrent miscarriage: A systematic review and meta-analysis. J Reprod Immunol. 2017;123:78-87.
19. Jiang X, Chai J, Li X, Li Y, Fu S, Liu F, et al. Machine learning-based integration of antiphospholipid antibodies for predicting pregnancy outcomes in patients with recurrent spontaneous abortion: A multicenter prospective study. Clin Chem Lab Med. 2026. [Epub ahead of print].
20. Guerby P, Fillion A, O'Connor S, Bujold E. Heparin for preventing adverse obstetrical outcomes in pregnant women with antiphospholipid syndrome, a systematic review and meta-analysis. J Gynecol Obstet Hum Reprod. 2021;50(2):101974.
21. Ahn Y, Hawkins C, Pearson E, Kubler P. Diagnosis and management of antiphospholipid syndrome. Aust Prescr. 2024;47(6):179.