

The Screening of Antiphospholipid Antibodies in Obstetric Antiphospholipid Syndrome-Like Events: A Regional Perspective

Onder M. ONEN¹, Fusun G. VAROL¹

Edirne, Turkey

ABSTRACT

OBJECTIVE: Obstetric antiphospholipid antibody syndrome is clinically recognized by adverse obstetric outcomes. To determine which antibody level best corresponds to the risk of these clinical outcomes is difficult. Obstetric antiphospholipid antibody syndrome-like adverse obstetric outcomes with single (n=108) and repeat (n=79) documented antiphospholipid antibody titers were evaluated.

STUDY DESIGN: Serum samples of 108 Obstetric antiphospholipid antibody syndrome cases and 50 healthy gestational matched controls with no history of thrombosis and congenital anomalies were subjected to testing for antiphospholipid antibodies with ELISA after the events. Of this obstetric antiphospholipid antibody syndrome group, only 79 cases underwent repeat testing within 12 weeks. Quantitative data were described by values and percentages at the levels of (>10) and (>40 U).

RESULTS: By one documented antiphospholipid test, the mean values of anticardiolipin, and anti-β2 glycoprotein1 (aβ2GP1) of obstetric antiphospholipid antibody syndrome versus controls were significantly different ($p<0.001$). Of 79 women who came for repeat sampling, a total number of women with persistent antiphospholipid antibody positivity was only 43 (54.43%). The number of obstetric antiphospholipid antibody syndrome cases with >40 U of antiphospholipid antibody was only 8 (18.60%).

CONCLUSION: Not all obstetric antiphospholipid antibody syndrome associated pregnancy morbidities may own high (>40U) antiphospholipid antibody titers, but low antiphospholipid antibodies (>10U) also accompany to this clinical picture. Obstetric antiphospholipid antibody syndrome should always be taken into account clinically prior to laboratory findings. Besides, long persistence of antiphospholipid-M positivity in these placenta-mediated disorders may make sense in terms of trophoblastic damage.

Keywords: Antiphospholipid antibody, IgM positivity, Obstetric antiphospholipid antibody syndrome
Gynecol Obstet Reprod Med 2020;26(3):162-165

Introduction

Repeated (>3) unexplained abortions (RUA), [<10th gestational week (GW)], unexplained fetal loss (UFL) (>10th GW), early-onset preeclampsia (EOPE) (<34th GW) are clinically recognized by adverse obstetric outcomes known as obstetric antiphospholipid antibody syndrome (oAPS) (1). A wide

¹ Department Obstetrics and Gynecology, Medical School, Trakya University, Edirne


Address of Correspondence: Fusun G. Varol
Trakya University Medical School
Department Obstetrics and Gynecology,
22030 Edirne, Turkey
fgvarol@yahoo.com

Submitted for Publication: 01.02.2019

Revised for: Publication: 30.03.2019

Accepted for Publication: 11.04.2019

ORCID IDs of the authors: OMO:0000-0001-9442-7475,
FGV: 0000-0003-1918-4746

Quick Response Code:	Access this article online
	Website: www.gorm.com.tr
	e-mail: info@gorm.com.tr
	DOI:10.21613/GORM.2018.905

How to cite this article: Onen OM, and Varol FG. The Screening of Antiphospholipid Antibodies in Obstetric Antiphospholipid Syndrome-Like Events: A Regional Perspective. *Gynecol Obstet Reprod Med.* 2020; 26(3):162-165

range of the antiphospholipid (aPL) antibodies (lupus anticoagulant (LA), anticardiolipin (aCL), and anti-β2 glycoprotein1 (aβ2GP1) may trigger the placenta-mediated disorders. However, to determine which antibody level best corresponds to the risk of these clinical outcomes is difficult (1). In APS, at least one clinical manifestation associated one persistent laboratory criterion with a threshold positivity (>40 GPL U/mL; MPL U/mL) was defined by International Society on Thrombosis and Haemostasis subcommittee (1-6).

There are several subjects under debate. The oAPS cases with low aPL levels have been reported to be thrombotic as well as with obstetrical complications (2,3). On the other hand, predominant single aPL positivity has been thought of as being characteristic of oAPS (4,5). Meanwhile, aCL-M antibodies have been reported as a risk factor for any placenta-mediated complications (6). Furthermore, the relationship between fetal loss and isolated IgM aCL as well as with low titer IgG aCL and the importance of aβ2GP1 antibodies would need more research to be clarified (7).

This case-control study evaluated regional oAPS-like cases at the levels of 10 U positivity for the assay as well as with a 40 U, a diagnostic criterion in APS. Low aPL titer was described as between 10-40 U. Besides, under the light of a

mini-review of the literature, oAPS-like adverse obstetric outcomes (n=108) with single documented aPL titer especially with IgM positivity were evaluated.

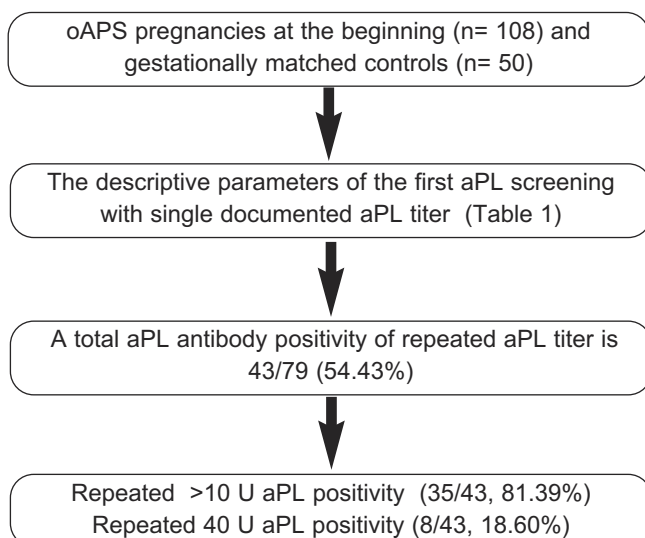
Material and Method

This prospective case-control study investigated a total of 108 consecutive patients [RUA (n=55), UFL (n=14), EOPE (n=39)] who were referred to the Department of Obstetrics and Gynecology at Trakya University over one-and half-year of period (1. March 2013 - 30. November 2014). This study was approved by local Ethics Committee (2013/09). The study was conducted in accordance with the Declaration of Helsinki and the patients' informed consent was obtained.

Serum samples of 108 oAPS cases and 50 healthy gestational matched controls with no history of thrombosis and congenital anomalies were subjected to testing for aPL antibodies (aCL-M, aCL-G, aβ2GP1-M, and aβ2GP1-G) with standardized an enzyme-linked immunosorbent assay (ELISA, DiaMetra, DCM114-6). The >10 U (IgG; GPL U/mL, IgM; MPL U/mL) is considered to be positive. Patients with obvious connective tissue disease and severe infection were excluded. Of this oAPS group, only 79 cases underwent repeat testing within 12 weeks. The flow chart for the study was shown in figure 1.

Quantitative data are described by values and percentages, mean±SD, median (minimum-maximum). Statistical evaluation was done with Student-T test, Chi-Square test ($p<0.05$).

Figure 1: The flow chart for the study



Results

The population characteristics and the descriptive parameters of aPL antibodies

One hundred eight oAPS and 50 controls were shown to be comparable in terms of age, Body Mass Index (BMI), gravidity, smoking and location ($p>0.05$) (Table 1). The descriptive

parameters of the aPL antibodies (aCL-M, aCL-G, aβ2GP1-M, aβ2GP1-G) by one documented aPL test were shown in table I. The mean values of aCL-M and aβ2GP1-M of oAPS cases versus controls were significantly different ($p<0.001$).

Table I: The population characteristics of single documented antiphospholipid titer screening in the obstetric antiphospholipid antibody syndrome-like events versus controls

	oAPS n=108	Control n=50	p
AGE			
<18	1 (0,9%)	1 (2%)	
18-35	82 (75.9%)	45 (90%)	
>35	25 (23.1%)	4 (8%)	0.066
BMI (kg/m ²)			
<18,5	2 (1.8%)	2 (4%)	
18,5-30	95 (87.9%)	44 (88%)	
>30	11 (10.1%)	4 (8%)	0.672
Trakya Region	108 (100%)	50 (100%)	
Family History	15 (13.8%)	5 (10%)	0.494
aCL-G			
Mean±SD	4.42±8.32	2.66±2.35	0.144
Median (Min-Max)	3.1 (0.5-30.8)	2.08 (0.85-6.94)	
aCL-M *			
Mean±SD	16.43±16.32	4.88±1.64	0.000
Median (Min-Max)	9.25 (1.3-74)	4.76 (2.3-8.71)	
aβ2GP1-G			
Mean±SD	3.81±7.32	3.19±0.85	0.554
Median (Min-Max)	1.95 (0.5-231)	3.1 (2.1-6.8)	
aβ2GP1-M *			
Mean±SD	3.83±5.03	1.83±0.49	0.006
Median (Min-Max)	2 (0.1-25.6)	1.7(1-3)	

aCL-G anticardiolipin-G, aCL-M anticardiolipin-M, aβ2GP1-G: Anti-β2glycoprotein1-G, aβ2GP1-M: Anti-β glycoprotein1-M

* p at significance

Evaluation the number of repeat aPL antibody positivity in oAPS-like adverse obstetric outcomes

According to the International Society on Thrombosis and Haemostasis subcommittee recurrent testing of aPL antibodies is needed for the definition of oAPS because of false positives at single testing. Of 79 women who came for repeat sampling, a total number of women with persistent aPL antibody positivity (>10U and 40U) was 43 (54.43%). The distribution of persistent (>10 U) aCL-G, aCL-M, aβ2GP1-G, aβ2GP1-M positivity was presented in table II. The number of oAPS cases with 40 U of aPL antibody was only 8 (18.60%) (Table III).

The vast distribution of aPL antibody positivity at >10 U was seen in RUA group (n=26) with aCL-G (n=3; 11.53%), aCL-M (n=19; 73.07%) and aβ2GP1-M (n= 4; 15.38%). The aCL-M positivity came into prominence at both levels of >10 and 40 U in all oAPS cases. Neither was positive in the controls.

Table II: Total number of patients with a titer more than 10 (n=35) with repeated antiphospholipid antibody positivity

aPL >10	RUA n=26 (%)	UFL n=4	EOPE n=5	Control
aCL-G	3 (11.5)	0	0	0
aCL-M	19 (73.07)	4(100)	5(100)	0
aβ2GP1-G	0	0	0	0
aβ2GP1-M	4(15.38)	0	0	0

RUA: Repeated (>3) unexplained abortions, UFL: Unexplained fetal loss, EOPE: Early-onset preeclampsia

Table III: Total number of patients with a titer more than 40 (n=8) with repeated antiphospholipid antibody positivity

aPL >40	RUA n=4	UFL n=2	EOPE n=2	Control
aCL-G	0	0	0	0
aCL-M	3	2	2	0
aβ2GP1-G	1	0	0	0
aβ2GP1-M	0	0	0	0

RUA: Repeated (>3) unexplained abortions, UFL: Unexplained fetal loss, EOPE: Early-onset preeclampsia

Discussion

This case-control study regionally evaluated aPL antibodies at two-titer levels (>10 and >40U) among oAPS-like cases. The total incidence of aPL antibodies among oAPS population was found as 54.43%. The majority (n=35; 81,39 %) of these adverse obstetric outcomes demonstrated aPL-M antibody positivity at >10 U. The number of the oAPS population with high titer (>40 U) was 8 (18.60%). This study also supports that oAPS like adverse obstetric outcomes (n=108) may have single documented aPL antibody titer with dominant IgM positivity. In terms of treatment, clinical manifestations should be taken into account before the antibody titer.

Obstetric antiphospholipid antibody syndrome is clinically recognized by aPL-associated adverse obstetric outcomes such as repeated unexplained abortions, unexplained fetal loss, early-onset preeclampsia (1). aPL-altered trophoblast dysfunction may cause ischemic placental dysfunction associated with fetal growth restriction, preeclampsia, premature birth, and intrauterine death. A direct causal association between aPL antibodies and pregnancy loss has been shown in murine models (8). The prevalence of oAPS varies according to the population and methodology studied along with low prevalence in normal pregnancies (9). However, the clinical risk of low aPL titers is still contradictory ranging from good outcomes mostly and to poor obstetric outcomes just like carrying medium-high titers (1,3,10,11).

Obstetric antiphospholipid antibody syndrome cases with low aPL levels have been also reported to be thrombotic as well as with obstetrical complications (2,3). Even though data implying that aPL antibodies may activate pregnancy loss through non-thrombotic mechanisms, pregnancies with purely

oAPS are at risk for thrombotic complications (12). However, APS pregnancies with high positive aPL titers are at the greatest risk group for obstetric morbidities (11). Among our oAPS population the only six patients (18.60%) showed persistent aCL-M positivity at >40 U. Predominant single aPL positivity was defined as being characteristic of oAPS (4,5). Significant differences at aPL titers especially aCL-M and aβ2GP1-M between oAPS cases and controls by one documented aPL tests were observed in our study too.

The perceived association between high aCL-G titers and fetal death is well recognized, especially with a previous history of fetal death (11). This correlation is so strong that even a single fetal death is considered as sufficient clinical criteria for APS (1). High levels of aβ2GPI and aCL were also shown to be associated with stillbirth (13). However, the debate on aCL and aβ2GPI titers among patients with fetal death, as well with recurrent early miscarriages have not been concluded yet.

A meta-analysis showed that aCL-M s were associated with late recurrent fetal loss in women without autoimmune disease (7). Our study also supported the finding that being positive for aCL-M was a risk factor in oAPS-like events. Even in women without autoimmune disease or hereditary thrombophilia, autoantibodies directly increase the risk of recurrent fetal loss and adverse obstetric outcome (14). Although aCL-M tends to give false-positive results, particularly in the low-positivity, being positive for aCL-M antibodies was found as a risk factor for any placenta-mediated complication (1,6). The addition of aβ2GP1 antibody may help clarify the results in the presence of any doubt of aCL-M false-positivity (1). But more data on the relationship between fetal loss and isolated IgM aCL as well as with low titer IgG aCL and the importance of aβ2GP1 antibodies would be useful (7).

The association between various aPLs and placenta mediated pregnancy complications are inconsistent and the literature examining this association is often underpowered (15). It is currently not clear to establish a significant link between aβ2GP1 antibodies and pregnancy related morbidity or adverse pregnancy outcomes. Particular attention should be exerted when establishing a diagnosis of APS based on the presence of any aPL antibody, particularly aβ2GP1 antibodies, in the setting of late pregnancy complications (15). On the other hand, aPL does not seem to be associated with infertility and treatment approach does not improve the outcomes in infertile patients with aPLs (16). However, moderate-to high level aCLs are found to be associated with preeclampsia (17).

As a consequence, not all oAPS associated pregnancy morbidities may own high (>40U) aPL antibody titers, but low aPL antibodies also may coincide to the clinical picture. Therefore, oAPS should always be taken into account clinically prior to laboratory findings. The treatment with low molecular weight heparins and low dose aspirin should be supported in the oAPS pregnancy morbidities even with low

aPL titer (18). In the near future longitudinal cohort studies with comprehensive public aPL data of certified laboratories can shed light on this issue. Those properly chosen oAPS cases will become candidates of antithrombotic treatment in the next pregnancies.

Acknowledgements: We thank Muzaffer Demir MD, Professor of Hematology, for his fruitful discussion of the manuscript. We also convey our gratitude to the teams of the Department Preventive Medicine and Biostatistics, School of Medicine, Trakya University.

Financial Disclosure: The authors have no financial relationship relevant to this article to disclose.

Conflict of interest: There are no conflict of interest.

Ethics Committee Approval: 2013/09

Author contribution: ÖÖ: Collects and analyzes data.

FGV: Analyzes data and writes the article.

References

- 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 (APS). *J Thromb Haemost.* 2006;4(2):295-306. DOI: 10.1111/j.1538-7836.2006.01753.x
- Roubey RA. Autoantibodies to phospholipids-binding-plasma proteins: a new view of lupus anticoagulants and other "antiphospholipid" antibodies. *Blood.* 1994;84(9):2854-67 PMID:7949161
- Mekinian A, Loire-Berson P, Nicaise-Roland P, Lachassinne E, Stirmemann J, Boffa MC, et al. Outcomes and treatment of obstetrical antiphospholipid syndrome in women with low antiphospholipid antibody levels. *J Reprod Immunol.* 2012;94(2):222-6 DOI:10.1016/j.jri.2012.02.004
- Boffa MC, Boinot C, De Carolis S, Rovere-Querini P, Arousseau MH, Allegri F, et al. Laboratory criteria of the obstetrical antiphospholipid syndrome. Data from a multi-centric prospective European women cohort. *Thromb Haemost.* 2009;102(1):225-8. DOI:10.1160/TH09-01-0043
- de Laat B, Pengo V, Pabinger I, Musial J, Voskuyl AE, Bultink IE, et al. The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. *J Thromb Haemost.* 2009;7(11):1767-73. DOI: 10.1111/j.1538-7836.2009.03588.x
- Bouvier S, Cochery-Nouvellon E, Lavigne-Lissalde G, Mercier E, Marchetti T, Balducci JP, et al. Comparative incidence of pregnancy outcomes in treated obstetric antiphospholipid syndrome: the NOH-APS observational study. *Blood.* 2014;123(3):404-13. DOI: 10.1182/blood-2013-08-522623
- Opatrny L, David M, Kahn SR, Shrier I, Rey E. Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a meta-analysis. *J Rheumatol.* 2006;33(11):2214-21. PMID:17014001
- Drakeley AJ, Quenby S, Farquharson R. Mid-trimester-loss-appraisal of a screening protocol. *Hum Reprod.* 1998;13(7):1975-80. DOI: 10.1093/humrep/13.7.1975
- Galarza-Maldonado C, Kourilovitch MR, Pérez-Fernández OM, Gaybor M, Cordero C, Cabrera S, et al. Obstetric antiphospholipid syndrome. *Autoimmun Rev.* 2012;11(4):288-95. DOI: 10.1016/j.autrev.2011.10.006
- Levy RA, Dos Santos FC, de Jesús GR, de Jesús NR. Antiphospholipid antibodies and antiphospholipid syndrome during pregnancy: diagnostic concepts. *Front Immunol.* 2015;6:205-9. doi: 10.3389/fimmu.2015.00205
- Simchen MJ, Dulitzki M, Rofe G, Shani H, Langevitz P, Schiff E, et al. High positive antibody titers and adverse pregnancy outcome in women with antiphospholipid syndrome. *Acta Obstet Gynecol Scand.* 2011;90(1):1428-33. doi: 10.1111/j.1600-0412.2011.01236.x
- Gris JC, Bouvier S, Molinari N, Galanaud JP, Cochery-Nouvellon E, Mercier E, et al. Comparative incidence of a first thrombotic event in purely obstetric antiphospholipid syndrome with pregnancy loss: the NOH-APS observational study. *Blood.* 2012;119(11):2624-32. DOI: 10.1182/blood-2011-09-381913
- Silver RM, Parker CB, Reddy UM, Goldenberg R, Coustan D, Dudley DJ, et al. Antiphospholipid antibodies in stillbirth. *Obstet Gynecol.* 2013;122(3):641-57. doi: 10.1097/AOG.0b013e3182a1060e
- Mumusoglu S, Beksaç MS, Ekiz A, Ozdemir P, Hascelik G. Does the presence of autoantibodies without antibodies and hereditary thrombophilia have an effect on recurrent pregnancy loss? *J Matern Fetal Neonatal Med.* 2016;29(14):2352-7. doi:10.3109/14767058.2015.1085964.
- Abou-Nassar K, Carrier M, Ramsay T, Rodger MA. The association between antiphospholipid antibodies and placenta mediated complications: a systematic review and meta-analysis. *Thromb Res.* 2011;128(1):77-85. doi: 10.1016/j.thromres.2011.02.006.
- de Jesus GR, Agmon-Levin N, Andrade CA, Andreoli LC, Highizola CB, Porter TF, et al. 14th International Congress on Antiphospholipid Antibodies Task Force Report on Obstetric Antiphospholipid Syndrome. *Autoimmun Rev.* 2014;13(8):795-813 doi: 10.1016/j.autrev.2014.02.003.
- do Prado AD, Piovesan DM, Staub HL, Horta BL. Association of anticardiolipin antibodies with preeclampsia: a systematic review and meta-analysis. *Obstet Gynecol.* 2010;116(6):1433-43. doi: 10.1097/AOG.0b013e3181fe02ec.
- Schreiber K, Radin M, Sciascia S. Current insights in obstetric antiphospholipid syndrome. *Curr Opin Obstet Gynecol.* 2017;29(6):397-403. doi: 10.1097/GCO.0000000000000406.