Article presented at 2nd International Nineveh Conference on Medical Sciences (2nd INCMS 2023)

Original Article

The Association between Antiphospholipid and Coagulation in Pregnant Women with Blood Clotting

Dabah Dakil Awad[1], Iktefa Abdul Hameed Mohammed Saeed[1], Maha El. Jasim[2]

[1]Department of Biology, College of Education for Women, Tikrit University, Tikrit, Iraq, [2]Department of Pharmacy, Institute of Technical Al-Dour, Northern Technical University, Mosul, Iraq

Downloaded from http://journals.lww.com/mjby by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 11/24/2024

Abstract

Background: Antiphospholipid syndrome is a systemic hematological autoimmune disease characterized by a hypercoagulable state, which is associated with vascular thrombosis and/or obstetric morbidity characterized by miscarriage, fetal death, and/or premature birth. **Objective:** This research study aimed to estimate anticardiolipin [ACL; immunoglobulin (Ig)G and IgM] and lupus anticoagulant (LA) in pregnant women who have blood clots or who have previously miscarried and fetal intrauterine mortality. **Materials and Methods:** This study included 90 women, 60 of whom were pregnant women who had blood clots or had previously miscarried, as well as 30 nonpregnant women as a control group, aged from 20 to 41 years, from October 2022 to April 2023. The patients were referred to the Department of Gynecology and Obstetrics at a Teaching Hospital, Salahuddin. LA, ACL IgM, and ACL IgG were detected in serum by the enzyme-linked immunosorbent assay. **Results:** This study found that pregnant women have higher ACL IgM and IgG levels than nonpregnant women. The mean concentration (mean ±standard error) for ACL IgM was 23.43 ± 1.4, whereas ACL IgG was 33.11 ± 1.28, which is in contrast with the control group of ACL IgM (2.99 ± 0.22) and ACL IgG (3.51 ± 0.29). The differences were statistically significant ($P = 0.0001$). A significant increase in LA in pregnant women was 37.77 ± 1.14, whereas the control group had a lower mean amount of LA was 334.42 ± 1.05. Statistically significant differences were observed ($P \leq 0.05$). **Conclusion:** Elevated levels of ACL and LA are the main cause of spontaneous recurrent abortions in women.

Keywords: ACL IgG, ACL IgM, LA, pregnant women, recurrent spontaneous abortion

Introduction

One of the most common clinical problems faced by doctors and patients, especially pregnant women, is abortion because of its psychological, social, and health complications that affect the pregnant woman and her family.[1] Pregnancy increases the risk of thrombosis four to five times. About 75%–80% of pregnancy-related clots are venous, and 25% are arterial. The main increase in risk is hypercoagulability. Both genetic and physiological factors can increase the risk of gestational thrombosis including permanent vascular damage, thrombotic disability,[2] and risk factors for venous thromboembolism during pregnancy. The strongest risk factor for venous thromboembolism during pregnancy is a history of blood clots. One of the risk factors that pregnant women may face is antibodies to phospholipids. Antiphospholipid antibodies, such as lupus anticoagulants (LAs) and antibodies to cardiolipin, are antibodies directed against negatively charged phospholipids. They have received great attention in the past decades to a group of charged phospholipids and have been associated with their presence with a thrombolytic tendency, resulting in a variety of clinical symptoms in pregnant women. The presence of these antibodies may have consequences for pregnancy loss.[3]

Retrospective reports demonstrated an association between LAs or antiphospholipid anticoagulants with stillbirths, possibly due to a blood clot in the placenta.[4]

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_926_23

Address for correspondence: Dabah Dakil Awad, Department of Biology, College of Education for Women, Tikrit University, Tikrit 34001, Iraq.
E-mail: dabyah.da@ntu.edu.iq

Submission: 06-Jul-2023 **Accepted:** 27-Sep-2024 **Published:** 21-Nov-2024

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Awad DD, Saeed IAHM, Jasim ME. The association between antiphospholipid and coagulation in pregnant women with blood clotting. Med J Babylon 2024;21:S272-5.

Downloaded from http://journals.lww.com/mjby by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 11/24/2024

Antiphospholipid syndrome (APS) generally refers to pregnancy complications including pre-eclampsia, placental malfunction, retarded fetal growth, and often repeated miscarriages.[2,4] The main cause of these complications is reported to be due to anticardiolipin (ACL) antibodies (ACAs) present in the circulating blood of these patients ACAs lead to thrombosis after binding with phospholipids by inhibiting the release of gonadotropins as well as placental anticoagulant proteins.[4,5] Although the exact mode of action of the placental-associated thrombosis of these antibodies is not clear, it is suggested in the literature that these antibodies may cause local acute inflammatory responses and neutrophil infiltration, which ultimately ends up with fetal loss.[6]

Materials and Methods

Study population and design

This study included 90 pregnant women, 60 of whom were threatened with miscarriage, and 30 nonpregnant women as a control group. The patients were referred to private clinics in the city of Tikrit and the Tikrit Teaching Hospital from October 2022 to April 2023 with their ages ranging from 20 to 41 years enrolled in the study. Before the study, a complete physical examination of all the candidates was carried out by a registered physician. In the first step of the screening, women with a previous history of any digestive, renal, endocrine, rheumatic, or nutritional disease including malnutrition (weight loss, low body mass index, or special dietary habits), undergoing a surgical process, blood donation or blood transfusion within 6 months, strenuous exercise, or heavy manual labor were excluded. Similarly, candidates with hypertension (systolic pressure ≥150 mm Hg and/or diastolic pressure ≥95 mm Hg) or heavy smokers (smoking >20 cigarettes/day) were also excluded from the study. In the second step of screening, candidates with positive hepatitis B surface antigen, hepatitis C by doctors in patient especially pregnant women.

Data collection

In a clinical serology laboratory, by vein puncture, 5 mL of blood was drawn and allowed to clot for 30 min at room temperature. After centrifugation for 10 min at 3400 rpm, serum was collected in the sterile tubes, and the samples were analyzed for LA, ACL immunoglobulin (Ig)M, and ACL IgG.

LA, ACL IgM, and ACL IgG analysis by enzyme-linked immunosorbent assay (ELISA)

ACL level was measured by an enzyme-linked immunoassay test using Orgentec kits (ORGENTEC Diagnostika, Mainz, Germany). Standardization and quantitation of results are based on the work of Loizou *et al.*,[7] using the polyclonal "Harris" standards.[8] Anti-β2-GP1 antibodies were assayed with commercial ELISA kits (QUANTA Lite™ β2 GP1, INOVA Diagnostics Inc., San Diego, CA, USA) for semi-quantitative determination. The cutoff value was determined to be 8 U/mL as defined by the manufacturer.[9,10] As described previously,[9,10] LA was assayed in fresh plasma samples using validated lupus ratio (LR) tests. Two LR tests were performed, one based on the activated partial thromboplastin time and the other based on the Russell viper venom time. The LR tests were performed on a 1:1 mixture of patient plasma and pooled normal plasma. For each of the LR tests, two coagulation times were measured, one with a reagent with a low phospholipid concentration and the other with a high phospholipid concentration. The ratio between two coagulation times (low phospholipid/high phospholipid concentration) was divided by the corresponding ratio obtained with pooled normal plasma. The final ratio is defined as the LR of that patient's plasma.[10]

Statistical analysis

The statistical analysis system Statistical Analysis System (2018) program (SAS. Inst. Inc. Cary. N.C. USA) was used to detect the effect of different groups (patients and control) on the study parameters. *T* test was used to significantly compare the means between the groups. The chi-square test was used to significantly compare between percentages (0.05 and 0.01 probability) in this study.

Ethical approval

The study was conducted following the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval from patients before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to document number 13105 on October 5, 2022.

Results

This study compares the levels of antiphospholipid antibodies, specifically ACL IgM and ACL IgG.

The findings of this study indicate a notable rise in the levels of ACL IgM and ACL IgG among pregnant women. The mean concentration, represented as mean ± standard error (SE), for ACL IgM was 23.43 ± 1.4, and for ACL IgG was 33.11 ± 1.28. In comparison, the control group exhibited lower mean concentrations, with ACL IgM at 2.99 ± 0.22 and ACL IgG at 3.51 ± 0.29. These differences were statistically significant, with a *P* value of 0.0001 [Table 1; Figure 1].

The findings of this study indicate a notable rise in the levels of LA among pregnant women [Table 2]. The mean concentration, represented as mean ± SE, for LA, was 37.77 ± 1.14. In comparison, the control group exhibited lower mean concentrations, with LA at 334.42 ± 1.05. These differences were statistically significant, with a *P* value of ≤0.05 [Figure 2].

Downloaded from http://journals.lww.com/mjby by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 11/24/2024

Table 1: Comparison between pregnant women and control groups in anticardiolipin (ACL) IgM and ACL IgG

Groups	Mean + SE	
	ACL IgM	ACL IgG
Pregnant women	23.43 ± 1.4	33.11 ± 1.28
Control	2.99 ± 0.22	3.51 ± 0.29
T test	3.316**	4.084**
P value	0.0001	0.0001

**$P \leq (0.01)$

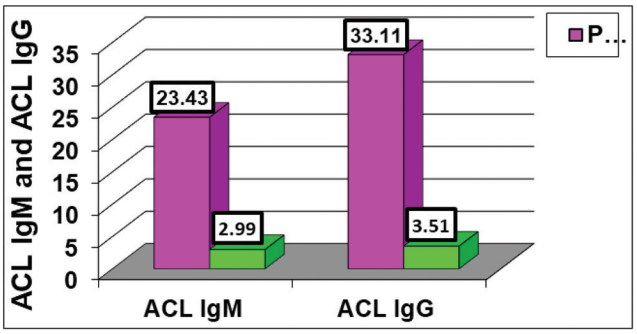

Figure 1: Comparison between pregnant women and control groups in anticardiolipin (ACL) IgM and ACL IgG

Table 2: Comparison between pregnant women and control groups in lupus anticoagulant (LA)

Groups	Mean + SE of LA
Pregnant women	37.77 ± 1.14
Control	34.42 ± 1.05
T test	3.193*
P value	0.041

**$P \leq 0.05$

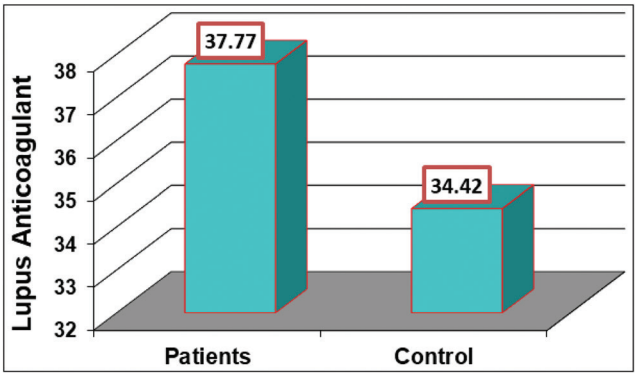

Figure 2: Comparison between patients and control groups in lupus anticoagulant

Discussion

These patterns can also be found at the same time as ACL-sharing studies. IgG,[11] thrombosis, and abortions are frequent fetal loss and thrombocytopenia, especially at high levels, as the disease IgA pattern appears to have. IgM reflects a small risk of thrombosis and is often associated with drug-induced antiphospholipid antibodies or co-production with sepsis. Although the IgG pattern is most people with primary APS who have frequent thrombosis or miscarriages, other patterns of ACL can also be associated with thrombosis, with ACLs being the second-most frequently associated with the role of both IgG. Then, IgA type III—most people with positive levels of ACL have no efficacy against LAs, most people with LA who do not have positive levels of ACL share LA with venous thrombosis,[12] and most people with antiphospholipid antibodies share arteriovenous thrombosis, whereas ACL share both arteriovenous and venous thrombosis—ACL type A type BB endotoxins GL factor II is dependent on the effectiveness of this factor GL or GL1 potential prothrombin[13] is the most important antibody to LA. The presence of IgG and IgM antibodies against cardiolipin and LA is known to be associated with coagulation and pregnancy loss, making the presence of these antibodies necessary for patients' classification as APS Association and pathogenesis of ACAs in patients with systemic lupus erythematosus as well as in the patients suffering from unexplained spontaneous abortions is controversial. Although a weak correlation of adverse effects of ACAs has been found in a large obstetric group, Love *et al.*[14] Although substantial knowledge has been gained regarding the clinical manifestations of APS, the role of antiphospholipid antibodies in the pathogenesis of this disorder remains poorly understood.

A literature survey indicates that the Sapporo classification criteria for APS, first proposed in 1999,[15] updated at the Eleventh International Congress on Antiphospholipid Antibodies in Sydney in 2006[16] are most often used in practice as diagnostic criteria for these antibodies. We have, therefore, interpreted our results as defined by the above-mentioned preliminary or modified Sapporo criteria.

High LA is a cause of many repeated miscarriages where a scientific study confirmed a close link between LA and RPLs causing[17] choroidal insufficiency or pre-retrograde growth and delayed intrauterine growth due to antithrombotic choroembolic protein, which is an anticoagulant annexin.[18-20] The presence of LA is a risk factor for recurrent miscarriage, especially in the first and second trimesters. Pregnant women with lupus are more likely to have complications of pregnancy than those without it.[21] High levels of lupus and ACL antagonists known as the top two antiphospholipid antibodies closely related to pregnancy loss[22] have been found to correspond to our results. A strong correlation has been reported between LA, ACL, the number of abortions, and a high level of LA positive correlation with increased gestational age.[23-25]

Downloaded from http://journals.lww.com/mjby by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 11/24/2024

Conclusion

Based on the results of the current study, it is concluded that elevated levels of APAs are the main cause of spontaneous recurrent abortions in women. ACL antibody is found to be the most important factor for recurrent abortion. In addition, women with negative ACL tested positive for another antiphospholipid antibody-like APS, which may be involved in recurrent abortion. Thus, suggesting that these APA assays are an effective diagnostic tool for the detection of recurrent spontaneous abortions.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Folkeringa N, Brouwer JLP, Korteweg FJ, Veeger NJ, Erwich JJH, Van Der Meer J. High risk of pregnancy-related venous thromboembolism in women with multiple thrombophilia defects. Br J Haematol 2007;138:110-6.
2. Musallam, ES, Yassin MM. Hemostatic Profile of Normal Pregnant Women in Gaza Strip. Doctoral dissertation, The Islamic University–Gaza; 2016. P. 55-60.
3. Al Jameil N, Tyagi P, Al Shenefy AI. Incidence of anticardiolipin antibodies and lupus anticoagulant factor among women experiencing unexplained recurrent abortion and intrauterine fetal death. Int J Clin Exp Path 2015;8:3204.
4. Hamid AM, Al-Rihawi W. The Usefulness of Investigating the Presence of Mobile Lupus Anticoagulant Antibodies in the Blood in Investigating the Possible Causes of the Occurrence of Recurrent and Idiopathic Miscarriages, an Unpublished Master's Thesis. Damascus: University of Damascus; 2000.
5. Carmo-Pereira S, Bertolaccini ML, Escudero-Contreras A, Khamashta MA, Hughes GRV. Value of IgA anticardiolipin and anti-β2-glycoprotein I antibody testing in patients with pregnancy morbidity. Ann Rheum Dis 2003;62:540-3.
6. Bu C, Zhang C, Li Z, Gao L, Xie Z, Cai G. Autoantibodies to plasminogen and tissue plasminogen activator in women with recurrent pregnancy loss. Clin Exp Immunol 2007;149:31-9.
7. Loizou SJA, McCrea JD, Rudge AC, Reynolds R, Boyle CC, Harris EN. Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): Standardization and quantitation of results. Clin Exp Immunol 1985;62:738.
8. Harris EN, Gharavi AE, Patel SP, Hughes GR. Evaluation of the anti-cardiolipin antibody test: report of an international workshop held 4 April 1986. Clin Exp Immunol 1987;68:215-22.
9. Schjetlein R, Sletnes KE, Wisløff F. A quantitative, semi-automated and computer-assisted test for lupus anticoagulant. Thromb Res 1993;69:239-50.
10. Jacobsen EM, Barna-Cler L, Taylor JM, Triplett DA, Wisløff F. The lupus ratio test–An interlaboratory study on the detection of lupus anticoagulants by an APTT-based, integrated, and semi-quantitative test. Thromb Haemost 2000;83:704-8.
11. Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. N Engl J Med 2002;346:752-63.
12. Shaikhomar OA, Ali ST. A comparative analysis of anticardiolipin, anti-B2-glycoprotein-1, and lupus anticoagulants in Saudi women with recurrent spontaneous abortions. J Pers Med 2022;13:2.
13. Hell L, Ay C, Posch F, Gebhart J, Koder S, Mackman N, *et al*. Low extracellular vesicle–associated tissue factor activity in patients with persistent lupus anticoagulant and a history of thrombosis. Ann Hematol 2019;98:313-9.
14. Love PE, Santoro SA. Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders: Prevalence and clinical significance. Ann Intern Med 1990;112:682-98.
15. Wendell A. Internationalconsensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Arthritis Rheum 1999;42:1309-11.
16. Franco C, Walker M, Robertson J, Fitzgerald B, Keating S, McLeod A, *et al*. Placental infarction and thrombophilia. Obstet Gynecol 2011;117:929-34.
17. Gezer S. Antiphospholipid syndrome. Disease-a-Month 2003;49:696-741.
18. Rasool ZS, Tiwari V. Biochemistry, Lupus Anticoagulant; 2019. p. 55-60 .
19. Arreola-Diaz R, Majluf-Cruz A, Sanchez-Torres LE, Hernandez-Juarez J. The pathophysiology of the antiphospholipid syndrome: A perspective from the blood coagulation system. Clin Appl Thromb Hemost 2022;28:10760296221088576.
20. Harris EN, Pierangeli SS. Revisiting the anticardiolipin test and its standardization. Lupus 2002;11:269-75.
21. Branch DW, Silver R, Pierangeli S, Van Leeuwen I, Harris EN. Antiphospholipid antibodies other than lupus anticoagulant and anticardiolipin antibodies in women with recurrent pregnancy loss, fertile controls, and antiphospholipid syndrome. Obstet Gynecol 1997;89:549-55.
22. Velayuthaprabhu S, Archunan G. Evaluation of anticardiolipin antibodies and antiphosphatidylserine antibodies in women with recurrent abortion. Indian J Med Sci 2005;59:347-52.
23. Mankee A, Petri M, Magder LS. Lupus anticoagulant, disease activity and low complement in the first trimester are predictive of pregnancy loss. Lupus Sci Med 2015;2:e000095.
24. Mari ZM, Smaism MF, Al-Hilli NM. Effect of obesity on androgen receptor and androgen levels in the serum of women with infertility in Babylon, Iraq. Med J Babylon 2023;20:433-6.
25. Mahmood OA. Maternal obesity and risk of fetal congenital abnormality. Med J Babylon 2019;16:364-6.