Serum Anti-Cardiolipin Antibodies among Women with Recurrent Abortions in Diyala Province

Abdul-Razak shafiq Hasan[•] Ph.D.

Abbas A. Al-Duliami [**] Ph.D.

Rafah O. A-Zubiadi[***] M.Sc.

Abstract

Background: Anticardiolipin antibodies (ACL) are heterogenous group of autoantibodies directed against negatively charged phospholipid and phospholipid binding proteins. They gained much attention due to their association with pregnancy failure, particularly recurrent fetal loss.

Objectives: To investigate the relevance of positive ACL antibodies with recurrent abortion among women in Diyala province and to explore the effect of certain co-factors.

Materials and methods: The study groups include, 45 pregnant women with previous history of recurrent abortion (mean age 29.3 ± 6.7) years; 60 pregnant women without previous abortion (mean age 28 ± 6.1) years; 50 non-pregnant women with history of recurrent abortion (mean age 29.7 ± 6) years and 60 non-pregnant women without history of previous abortion (mean age 31.5 ± 7.2) years. Required information were collected by personal interview. Detection of ACL-IgM and ACL-IgG were done using ELISA technique.

Results: The results showed that the seropositivity of ACL-IgM among pregnant women with recurrent abortion and without abortion was 30% and 3.3% respectively. While the seropositivity of ACL-IgM among non-pregnant women with previous abortion and those without abortion was 20% and 0% respectively. The ACL-IgG among non-pregnant women without previous abortion and those with previous abortion was 0% and 35% respectively.

Conclusion: Anticardiolipin antibodies may play as a cause of recurrent spontaneous abortion among women in Diyala province.

[•] College of Vet. Med. \Diyala University

[**] College of Education, Diyala University

[***] Baquba Technical Institute

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Introduction

Antiphospholipid antibodies are a heterogenous group of autoantibodies directed against negatively charges phosphlpids and phosphlipid binding proteins. They are associated with aterial and venous thrombosis, thrombocytopenia, pregnancy complications and reproductive autoimmune failure [1]. There are three primary classes of phospholpid autoantibodies; lupus anticoagulant, antibodies directed against specific molecule known as β2-glycoprotein I, and anticardiolipin [2]. Cardiolipin is synthesized in the mitochondria, and it is an important component of metabolically active cells of the heart and skeletal muscles [3,4]. Recurrent spontaneous abortion is a critical problem in which many factors play a role such as genetic, hormonal disorders, uterine factors, infections, environmental and immunologic factors [5]. ACL antibodies play an important role in reproductive failure by impairing embryonic implantation [6]. The association of ACL antibodies with thrombotic events and fetal loss providing it as more sensitive and specific marker for recurrent spontaneous abortion than lupus anticoagulent antibodies [7,8]. It has been demonstrated that 2-16% of wome with reproductive failure during the first trimester were ACL positive [9]. Furthermore, ACL positive antibodies are present in 20.9% of women with severe preeclmpsia, 30.9% of women with fetal loss, and 33% of those with placental abruption [10-12].

Materials And Methods

The present study was conducted in Baquba city for the period from 1 November/2007 to 15 March/ 2009. Subjects included in this study were chosen from those attending Baquba Teaching Hospital, Al-Batool Teaching Hospital for maternity and children, as well as three Primary Health Care centers in Baquba. The study groups include, 45 pregnant women with previous history of recurrent abortion (mean age 29.3 ± 6.7) years; 60 pregnant women without previous abortion (mean age 28 ± 6.1) years; 50 non-pregnant women with history of recurrent abortion (mean age 29.7 ± 6) years and 60 non-pregnant women without history of previous abortion (mean age 31.5 ± 7.2) years. Required information were collected by personal interview. Detection of ACL-IgM and ACL-IgG were done using ELISA

commercially available kits (Aeskulisa/ Germany). All data were statistically analyzed using the SPSS computer assisted program version 13.

Results

The ACL-IgG positivity rate among non-pregnant women without previous abortion was 6.7%, whereas, non of the pregnant women without history of abortion gave positive result. The difference between the two groups is statistically insignificant (P= 0.16), table (1).

Table (1): ACL-IgG positivity rate among pregnant and non-pregnant women without recurrent abortion.

ACL-IgG	No recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Non-pregnant	Pregnant			
	No.(%)	No.(%)			
Negative	56(93.3)	30(100)	0.2	4.9	0.02-1.84
Positive	4(6.7)	0(0)			
Total	60(100)	30(100)			

P= 0.16 [NS] CI: Confidence interval

Table (2): ACL-IgG positivity rate in pregnant and non-pregnant women with recurrent abortion.

ACL-IgG	Recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Non-pregnant	Pregnant			
	No.(%)	No.(%)			
Negative	13(65)	16(64)	1.0	**	0.31-3.5
Positive	7(35)	9(36)			
Total	20(100)	25(100)			

P= 0.94 [NS] CI: Confidence interval

Table (2) showed that the ACL-IgG positivity rate among pregnant and non-pregnant women who had previous history of recurrent abortion was 35% and 36% respectively, the difference between them was statistically insignificant (p=0.94).

The ACL-IgG positivity rate among non-pregnant women with previous history of recurrent abortion was significantly (P=0.004) higher compared to non-pregnant women without history of abortion (35% and 6.7%) respectively, table (3).

Table (3): Risk of ACL-IgG in non-pregnant women with and without recurrent abortion.

ACL-IgG	Recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Negative	Positive			
	No.(%)	No.(%)			
Negative	56(93.3)	13(65)	7.5	**	1.9-29.6
Positive	4(6.7)	7(35)			
Total	60(100)	20(100)			

P= 0.004 [S] CI: Confidence interval

Pregnant women with previous history of recurrent abortion had significantly higher (P=0.001) ACL-IgG compared to pregnant women without history of recurrent abortion, table (4).

Table (4): Risk of ACL-IgG in pregnant women with and without recurrent abortion.

ACL-IgG	Recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Negative	Positive			
	No.(%)	No.(%)			
Negative	30(100)	16(64)	35.1	**	4.1-298.2
Positive	0(0)	9(36)			
Total	30(100)	25(100)			

P= 0.001 [S] CI: Confidence interval

Among all pregnant and non-pregnant women who had positive history of recurrent abortion had significantly higher (P< 0.001) ACL-IgG positivity rate compared to those who had no history of recurrent abortion (35% and 4.4%) respectively, table (5).

Table (5): Risk of ACL-IgG in overall women with and without recurrent abortion.

ACL-IgG	Recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Negative	Positive			
	No.(%)	No.(%)			
Negative	86(95.6)	29(64.4)	11.9	**	3.67-38.36
Positive	4(4.4)	16(35.6)			
Total	90(100)	25(100)			

P< 0.001 [S] CI: Confidence interval

Table (6) showed that the difference in the ACL-IgM positivity rate in pregnant and non-pregnant women without recurrent abortion was statistically insignificant (P=0.37).

Table (6): ACL-IgM positivity rate among pregnant and non-pregnant women without recurrent abortion.

ACL-IgM	No recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Non-pregnant	Pregnant			
	No.(%)	No.(%)			
Negative	30(100)	29(96.7)	3.1	**	0.27-36.0
Positive	0(0)	1(3.3)			
Total	30(100)	30(100)			

P= 0.16 [NS] CI: Confidence interval

The ACL-IgM positivity rate among pregnant and non-pregnant women who had positive history of recurrent abortion was 30% and 20% respectively. The difference between the two groups was statistically insignificant (P=0.42), table (7).

Table (7): ACL-IgM positivity rate in pregnant and non-pregnant women with recurrent abortion.

ACL-IgM	Recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Non-pregnant	Pregnant			
	No.(%)	No.(%)			
Negative	24(80)	14(70)	1.7	**	0.46-6.3
Positive	6(20)	6(30)			
Total	30(100)	20(100)			

P= 0.42 [NS] CI: Confidence interval

Table (8) showed that non-pregnant women with positive history of recurrent abortion had significantly higher (P=0.012) ACL-IgM positivity rate compared to their counterpart without history of recurrent abortion.

Table (8): Risk of ACL-IgM in non-pregnant women with and without recurrent abortion.

ACL-IgM	Recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Negative	Positive			
	No.(%)	No.(%)			
Negative	30(100)	24(80)	16.2	**	1.87-140.4
Positive	0(0)	6(20)			
Total	30(0)	30(100)			

P= 0.012 [S] CI: Confidence interval

Similarly, pregnant women with positive history of recurrent abortion had significantly higher (P=0.025) ACL-IgM positivity rate as compare to pregnant women without history of recurrent abortion (30% and 3.3%) respectively, table (9).

Table (9): Risk of ACL-IgM in pregnant women with and without recurrent abortion.

ACL-IgM	Recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Negative	Positive			
	No.(%)	No.(%)			
Negative	29(96.7)	14(70)	12.4	**	1.36-113.4
Positive	1(3.3)	6(30)			
Total	30(100)	20(100)			

P= 0.025 [S] CI: Confidence interval

The ACL-IgM positivity rate among overall women with positive history of recurrent abortion was significantly higher (P< 0.001) compared to those without recurrent abortion, table(10).

Table (10): Risk of ACL-IgM in overall women with and without recurrent abortion.

ACL-IgM	Recurrent abortion		Odd ratio	Inverse Odd ratio	95% CI by Chi-method
	Negative	Positive			
	No.(%)	No.(%)			
Negative	59(98.3)	38(76)	18.6	**	2.33-149.2
Positive	1(1.7)	12(24)			
Total	60(100)	50(100)			

P< 0.001 [S] CI: Confidence interval

Discussion

The present study showed that the positivity rate of ACL IgG antibody among non-pregnant women without history of previous abortion was 6.7%, and that of pregnant women without history of recurrent abortion was (0%). These results are in agreement with previous studies [11]. However, these result are inconsistent with the previous studies which reported a positivity rate of ACL IgG antibody in women with normal pregnancy was ranged between 7%-17% [13,14].These controversial results probably due to residual antibodies of past positivity to ACL IgG antibody during the non-gestational period, and in any how it refers to past positivity to ACL IgM antibody [15,16].

These results found that the positivity rate of ACL IgG in pregnant women with history of recurrent spontaneous abortion was insignificantly higher than that in non-pregnant women . These result are consistent with the previous reports [17,18]. It has been reported that ACL IgG may be considered as a risk factor for pregnancy losses, Suggesting that women with high ACL IgG titers had up to 80% risk of current pregnancy loss [19]. Moreover, increase of spontaneous abortion, preeclampsia, fetal growth restriction and fetal death before 24 weeks of gestation have been observed in women with positive anticardiolipin IgG antibodies [20].

The ACL IgG positivity rate among non – pregnant women with history of fetal losses in the present study seems not unusual, since pregnant women with previous history of abortion had nearly similar positivity rate for ACL IgG. These antibodies may be remnants of previous pregnancies and / or previous abortions [21]. Affirming that, another study reported that the ACL IgG was raised upto 40% in non pregnant women with habitual abortion [22].

These results are clearly indicated the significant association between the presence of ACL IgG and the recurrent abortion among non pregnant women. women with anticardiolipin

IgG antibody are at an increased risk for thrombosis during pregnancy [23]. Moreover, women with underlying autoimmune diseases may have anticardiolipin antibodies even before they ever become pregnant [24].Therefore, non – pregnant women or those in the inter-gestational period who tested positive for ACL IgG should be advice to delay conception [16].

In present study, the risk that pregnant women with history of recurrent abortion to have ACL IgG positive was 35.1 times more than pregnant women with out history of recurrent abortion. Similar result have been obtained by other workers, who found that the presence of anticardiolipin antibody probably precedes the development of disease in non – pregnant women with recurrent abortion [25,26]. Furthermore, Women with anticardiolipin IgG antibody have an increased risk for venous thrombosis during pregnancy, particularly in the post-partum setting [27].

The significantly higher ACL IgM positivity rate in non-pregnant women with history of recurrent abortion compared to those without history of recurrent abortion is cnsistent with previous studies found that the levels of anticardiolipin IgM antibody may be increased during the abortion process [10,28].

The significant increase of ACL IgM in pregnant women with recurrent abortion compared to their counterpart is consistent with those reported by other workers [12]. Furthermore, womens with antiphospholipid syndrome and previous pregnancy loss found to have only anticardiolipin IgM antibodies without Lupus anticoagulant [29].

References

[1] Espinosa, G.; Cervera, R. ; Font, J. and Shoenfeld, Y. Antiphospholipid syndrome: pathogenic mechanisms. J. Autoimmun. Rev. 2003; 2:86–93.

[2] McIntyre, J. A.; Wagenknecht, D.R. and Faulk, W. P. Antiphospholipid antibodies: discovery, definitions, detection and disease. Prog. Ipid. Res. 2003; 42(3):176-237.

[3] Chicco, A.J. and Sparagna, G.C. Role of cardiolipin alteration in mitochondria dysfunction and disease. Am. J. physiol. Cell Physiol. 2007; 292: C33-C44.

[4]Houtkooper, R.H. and Vaz, F. M. Cardiolipin, the heart of mitochondrial metabolic cell. J. Mol. Life .Sci. 2008; 65: 2493-2506.

[5] Sulani, S.; Ferriani, R.A.; Santos,C.M. and Voltario, J.C. Immunological evaluation of patients with recurrent abortion. J. Reprod.Immunol. 2003; 56:111-121.

[6]Choudhury, S.R. and Knapp, L.A. Human reproductive failure I: immunological factors. J. Hum. Reprod. Update,2001; 7:113-134.

[7]Nam, Y.S.; Cha, K.Y.; Baek, J.Y.; Kim, N.K.; Kang, M.S. and Oh, D. A Study of Lupus Anticoagulants and Anticardiolipin Antibodies in Patients with Infertility and recurrent spontaneous abortion. Korean .J. Fertil. Steril. 2002; 29 (1): 29-35.

[8]Nielsen,H.S. and Christiansen, O. B. Prognostic impact of anticardiolipin antibodies in women with recurrent miscarriage negative for the lupus anticoagulant. J. Hum .Repro. 2005;20:1720–8.

[9]Seyed Mahmood Ghoraishian ; Seyed Meddi Klantar ; Seyed Mohammd Seyed Hasani and Mohammed Ghafourzadeh. Comparison of anticardiolipin antibody and antiphospholipid antibody in women with recurrent abortions. Iranian .J. Reproductive Medicine. 2006; 4(2): 77-79.

[10]Daboubi, M.K. Anticardiolipin antibodies in women with recurrent abortion. East. Mediterr. Health. J. 2001; 7: 95-99.

[11]Tsapanos, V.; Kanellopoulos, N. and Cardamakis, E. Anticardiolipin antibodies levels in healthy pregnant and non pregnant women. Arch. Gynecol. Obstet. 2000; 263: 111-115.

[12]Sikkema,J.M.; Franx, A.; Bruinse, H.W.; Van der Wijk, N.G.; de Valk, H.W. and Nikkels, P.G..Placental pathology in early onset preecclampsia and intra-uterine growth restriction in women with and without thrombophilia. Placenta, 2002; 23: 337-42.

[13]Ajami, A. and Khalilian, A. Prevalence of IgG anticardiolipin antibody in recurrent pregnancy . J. Rese. Biol. Sci. 2007; 2 (2):139-142.

[14]Vinatier, D.P.; Dufour, M.; Cosson, and Houpeau, T.L. Antiphospholipid syndrome and recurrent miscarriage. Eur. J. Obstet. Gynecol. Reprod. Biol . 2001; 96:37-50.

[15] Salmon, J.E. and Giradi, G. Antiphospholipid antibodies and pregnancy loss: a disorder of in flammation. J. Reprod. Immunol . 2008; 77(1):51-6.

[16] Miyakis, S.; Lockshin, M.D.; Atsumi, T.; Branch, D.W. and Brey, R.L. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J. Thromb. Headmost. 2006; (2006). 4: 295-306.

[17] Rai, R.S. Antiphospholipid syndrome and recurrent miscarriage. J. postgrad. Med. 2002; 48: 3-4.

[18] Velayuthaprabhu, S. and Archunan, G. Evaluation of anticardiolipin antibodies and antiphosphatidylserine antibodies in women with recurrent abortion. Indian. J. Med. Sci. 2005; 59: 347-52.

[19] Mitiracui, N.; Bergi, L.; Hizem, S.; Nsiri, B.; Finan, R.R. and Gris, J.C. Prevalence of antiphospholipid antibodies in early and late recurrent pregnancy loss. Eur. J. Obstest. Gynecol. Report. Biol. 2005; 119: 164-70.

[20] Carmona, F.; Font, J.; Azulay, M.; Creus, M.; Fabregues, F. and Cervera, R. Risk factors associated with fetal losses in treated antiphospholipid syndrome pregnancies: a multivariate analysis. Am. J. Reprod. Immunol. 2001; 46: 274-9.

[21] Branch, D.W. Antiphospholipid antibodies and fetal compromise. J. Thromb. Res . 2004; 114:415–418.

[22] Coulam, C.B. and Roussev, R.G. Correlation of NK cell activation and inhibition markers with NK cytoxicity among women experiencing immunologic implantation failure after in vitro fertilization and embryo transfer. J. Assist. Reprod. Genet. 2003;.20:58–62.

[23]Lima, F.; Khamashta, M.A. and Buchanan ,N.M. A study of sixty pregnancies in patients with the antiphospholipid syndrome. Clin. Exp. Rheumatol. 1996; 14: 131-136.

[24] Nash, M.J.; Camilleri, R.S.; Kunka, S.; Mackie, I.J.; Machin, S.J. and Cohen, H. The anticardiolipin assay is required for sensitive screening for antiphospholipid antibodies. J. Thromb. Haemost. 2004; 2:1077–1081.

[25] Nam, Y.S.; Cha, K.Y.; Baek, J.Y.; Kim, N.K.; Kang, M.S. and Oh, D. A Study of Lupus Anticoagulants and Anticardiolipin Antibodies in Patients with Infertility and recurrent spontaneous abortion. Korean .J. Fertil. Steril. 2002; 29 (1): 29-35.

[26] Neville, C. ;Rauch, J.; Kassis, J. ;Chang, E.R.; Joseph, L. ; Le Comte, M. and Fortin, P.R. Thrombo-embolic risk in patients with high titer anticardiolipin and multiple antiphospholipid antibodies. J. Thromb. Haemost. 2003; 90:108–15.

[27] Sebire, N.J.; Backos, M.; El –Gaddal, S.; Goldin, R.D. and Regan, L. Placental pathology, antiphospholipid antibodies, and pregnancy outcome in recurrent miscarriage patients. J. Obstet. Gynecol. 2003; 101:258-63.

[28] Meroni, P.L. and Riboldi, P. Pathogenic mechanisms mediating antiphospholipid syndrome. Curr. Opin. Rheumatol. 2001; 13:377-82.

[29] Wong, R.C.; Adelstein, S.; Gillis, D. and Favaloro, E.J. Development of consensus guidelines for anticardiolipin and lupus anticoagulant testing. Semin .Thromb. Hemost. 2005; 31:39–48.