

## Prevalence of Anticardiolipin Antibodies in Pregnant Women with Recurrent Miscarriage in Al-Hilla city

Hanan Selman Hesan  
Dentistry college – Babylon University  
Hanan1985@yahoo.com

### Abstract

In the present study determine the level of serum anticardiolipin (ACL) antibodies (ACL- IgM and ACL-IgG) in 30 pregnancies women who clinically diagnosed with recurrent miscarriage more than two times their aged between 21– 42 years, attending to Babylon hospital of maternity and children and 15 healthy pregnant women without previous miscarriage were included as control in the study which extended from April 2013 to October 2013. Blood samples and the required information regarded with maternal age and number of previous miscarriage were collected from both patients and healthy. It was shown from the results that 4 (13.34 %) cases of pregnant women with previous miscarriage were positive in ACL- IgM and 6 (20 %) cases of pregnant women with recurrent previous miscarriage give positive for ACL- IgG, while all result of test give negative in healthy pregnant women. An obvious significant differences at probability level ( $P < 0.05$ ) found when make comparison between ACL- IgM and ACL-IgG level in both patient and healthy.

**Key word:** anticardiolipin antibodies, recurrent miscarriage, risk factor

### الخلاصة

في الدراسة الحالية تم تحديد مستوى الأجسام المضادة IgM و IgG للشحوم المفسفرة القلبية لدى 30 امرأة حامل قد تعرضن إلى حالات إجهاض متكررة لأكثر من مرتين سابقة مشخصة سريريا تراوحت أعمارهن بين 21 – 42 سنة، من اللاتي راجعن مستشفى بابل للولادة والأطفال و 15 امرأة حامل بصحة جيدة لم يتعرضن إلى حالة إجهاض سابقة قد عدوا سيطرة في هذه الدراسة التي امتدت للفترة من نيسان 2013 لغاية تشرين الأول 2013 . جمعت عينات الدم والمعلومات المتعلقة بعمر الأم وحالات الإجهاض السابقة من النساء المرضي والأصحاء. أظهرت نتائج الفحص بان هناك 4 ( 13.34%) حالة بين النساء الحوامل المجهضات سابقا قد أعطت نتائج ايجابية لاختبار ACL- IgM في حين ظهرت 6 (20 %) حالات ايجابية لفحص ACL- IgG بين النساء المجهضات سابقا مقارنة بمجموعة الحوامل الأصحاء التي أعطت نتائج سلبية للاختبار. وجد بان هناك فروقا معنوية واضحة بمستوى احتمال ( $P < 0.05$ ) عند المقارنة بين مستوى ACL- IgM و ACL- IgG عند كل من النساء المرضي والأصحاء على حد سواء.

**الكلمات المفتاحية:** الشحوم المفسفرة القلبية، الاجهاض المتكرر، عوامل الخطورة.

### Introduction

The plasma membranes of mammalian cells are formed from phospholipids, anionic phospholipids (eg, phosphatidylserine) are found on the cytoplasm surface and neutral phospholipids (eg, phosphatidylcholine) predominate on the external surface. Membrane phospholipids participate in several important cellular functions including exchanging metabolites across membranes, transferring molecular signals and serving as a platform for the assembly of protein-lipid complexes (Bevers *et. al.*, 1999; Velayulhaprabhu and Archunan, 2005)). Complexes of negatively charged (anionic) phospholipids and endogenous plasma proteins provide epitopes recognized by natural autoantibodies (Arnout and Vermynen, 2003). A cardiolipin is a phospholipid or lipid molecule in blood, synthesized in mitochondria and it is an important component of metabolically active cells of the heart and skeletal muscles (Houtkooper and Vaz, 2008).

Plasma from normal individuals contains low concentrations of natural IgG autoantibodies of moderate affinity. Pathologic levels of autoantibodies reflect loss of tolerance and increased production of antibodies, these autoantibodies are called phospholipid or cardiolipin antibodies (Salazar *et. al.*, 2002) they are detected by enzyme linked immunosorbent assays (ELISAs) in which the negatively charged phospholipid is coated on plastic plates as substrate, the most commonly used phospholipid substrate is cardiolipin (Castro and Gourley, 2010). Anticardiolipin antibody syndrome is usually an acquired condition, therefore it can not transmit from mother to child. The levels of anticardiolipin antibodies IgM and IgG are often high in people with abnormal blood clotting, autoimmune disease lupus (SLE) or recurrent miscarriage (Ong *et. al.*, 2002). Recurrent miscarriage is usually defined as three or more consecutive, spontaneous miscarriages occurring in the first trimester in which many factors play a role such as maternal age, genetic, hormonal disorders, uterine factors, the infections, environmental and immunologic factors (Clarisa *et. al.*, 2005). A successful birth may or may not follow; about one half of recurrent miscarriages are unexplained, antiphospholipid syndrome is one of the known causes of first- and second-trimester recurrent miscarriage (Ruiz-Irastorza *et. al.*, 2010). Antiphospholipid syndrome (APS) is defined as the presence of anticardiolipin antibodies or lupus anticoagulant antibodies in association with three or more consecutive fetal losses before 10 weeks of gestation (Choudhury and Knapp, 2001), one or more unexplained intrauterine deaths after 10 weeks of gestation, one or more premature births before 34 weeks of gestation due to severe preeclampsia or impaired fetal growth. Many studies have shown the correlation between these autoantibodies and enhanced incidence of abortion in pregnant women rate from 0.2% to 2% is similar to the frequency in the general population. Antiphospholipid antibodies are present in 15 percent of women with recurrent miscarriage (Rai *et. al.*, 1995; Heilmann *et. al.*, 2003; Kareem, 2012; Zakarea *et. al.*, 2013). In Iraq there are number of studies to evaluate the relationships between the incidences of anticardiolipin with recurrent miscarriage (Jwad *et. al.*, 2006; Risan, 2011). So, the aim of study was to determine the prevalence of anticardiolipin antibody (ACA) in women with recurrent miscarriage in Al-Hilla city.

## **Material and Method**

### **1- Patients and healthy**

The present study was conducted in Babylon teaching hospital for maternity and children during April 2013 to October 2013 on 30 pregnant women who had suffered from two or more time of recurrent miscarriage and 15 healthy pregnant women as control. The patients age range between 21- 42 years. The required information regarded in maternal age and number of previously birth losses was collected by personal interview from the pregnant women.

### **2- Specimens**

Three ml of blood sample was collected from both groups of pregnant women, the whole blood samples were centrifuged for 5 minute at 3000 rpm. The sera kept frozen at – 20 C° until performed the test, all specimens will treated and reading in measuring of ACL- IgG and ACL- IgM by ELISA method in Chorus Trio (DISSE Diagnostica Senese – Monteriggioni- Italy) system at Merjan teaching hospital and compare the readings result with accompanied reference values according to instruction of manufacturer's company as follow:

<b>Normal</b>	<b>IgG &lt; 12 GPLU/mL ,</b>	<b>IgM &lt; 12 MPLU/mL</b>
<b>Doubtful:</b>	<b>IgG = 12 – 18 GPL U/mL,</b>	<b>IgM = 12 – 18 MPL U/mL</b>
<b>Positive:</b>	<b>IgG &gt; 18 GPL U/mL,</b>	<b>IgM &gt; 18 MPL U/mL</b>

MPL refers to IgM Phospholipid Units. One MPL unit is 1 microgram of IgM antibody. GPL refers to IgG Phospholipid Units. One GPL unit is 1 microgram of IgG antibody.

### 3- Statistical analysis:

Data were analyzed using SPSS (statistical package for social science). Mean, Standard deviation and t- test (P- value < 0.05) were used as statistical parameters in this work.

### Results

Table (1) showed the occurrence of ACL- IgM in both studied groups, there are 4 (13.34%) cases of pregnant women with recurrent miscarriage give positive to ACL-IgM while 26( 86.66 %) of them give negative result as compared with healthy pregnant women give negative.

**Table 1: Distribution of pregnant with recurrent miscarriage and healthy pregnant according to anticardiolipin IgM**

	<b>Studied groups</b>		<b>Total</b>
	<b>Pregnant with recurrent miscarriage %</b>	<b>Healthy pregnant %</b>	
<b>Anticardiolipin Ab IgM</b>	<b>Positive</b> 4 (13.34 )	---	<b>4</b>
	<b>Negative</b> 26 (86.66)	<b>15 (100)</b>	<b>41</b>
<b>Total</b>	<b>30</b>	<b>15</b>	<b>45</b>

Table (2) expressed that 6 (20 %) for pregnant women with recurrent miscarriage give positive result in testing sera to ACL-IgG while 24 (80 %) of them give negative result compared with healthy women.

**Table 2: Distribution of pregnant with recurrent miscarriage and healthy pregnant according to anticardiolipin IgG**

	<b>Studied groups</b>		<b>Total</b>
	<b>Pregnant with recurrent miscarriage %</b>	<b>Healthy pregnant %</b>	
<b>Anticardiolipin Ab IgG</b>	<b>Positive</b> 6 (20 )	---	<b>6</b>
	<b>Negative</b> 24 (80)	<b>15 (100)</b>	<b>39</b>
<b>Total</b>	<b>30</b>	<b>15</b>	<b>45</b>

Table (3) shows there is a significant differences (P < 0.05) when compared the level of ACL- IgM and ACL-IgG between the pregnant women with recurrent miscarriage and healthy pregnant women.

**Table 3: level of anticardiolipin antibodies IgM, IgG (PLU/mL) in sera of pregnant with recurrent miscarriage and healthy pregnant women**

		ACL- IgM	ACL- IgG
<b>Pregnant with recurrent miscarriage</b> <b>N. 30</b>	<b>M</b>	<b>19.844</b>	<b>27.0660</b>
	<b>SD ±</b>	<b>26.977</b>	<b>33.1580</b>
<b>Healthy pregnant</b> <b>N. 15</b>	<b>M</b>	<b>5.5000</b>	<b>5.4800</b>
	<b>SD ±</b>	<b>2.0410</b>	<b>2.3710</b>
<b>Significance</b>		<b>Significant</b> <b>P &lt; 0.05</b>	<b>Significant</b> <b>P &lt; 0.05</b>

**M= mean****SD= standard deviation**

## Discussion

Antibodies against cardrdiolipin belong to the group of antiphospholipid antibodies (APL) a heterogeneous group of autoantibodies directed against negatively charged phospholipids a component of biological membrane (Reddel and Krilis, 1999).The anticardiolipin ACL assay serves to assist in diagnosis of APL that is important for treatment and predication of thrombosis and recurrent miscarriage (Branch *et al.*, 2000; Cowchock, 1996).

The current study detected high level of serum anticardiolipin ACL-IgM 4 (13.34 %) among recurrent miscarriage women with significant difference as compared with healthy women tables (1, 3) and increasing in level of ACL- IgG 6 (20 %) with significant difference as compared with healthy women tables (2, 3). The persistence presence in plasma of medium to high level of IgG and IgM class anticardiolipin antibodies and or Lupus anticoagulant is associated with both recurrent pregnancy loss and venous thrombosis (Derksen, 2001). Many studies agreed with our results that are found a significant association between repeated miscarriage and presence of high level of ACL- Abs. for example in Iraq the worker Jawad *et. al.* 2006 found (17.6 %) the rate of ACL-Abs. in pregnant women with fetal losses, another study by Risan, 2011 found high titer of ACL-IgG is of clinical significance in identifying women at a risk of pregnancy loss then ACL-IgM antibodies. A study from Jordan in 2001, found that in a group of 26 women defined as habitual abortion, 19.23 % had positive ACL- Abs. as compared with control group (Daboubi, 2001). Similar findings were found in study carried out in Brazil, high level of ACL- IgM (41.1%) and (17.6%) for ACL- IgG (Spegiorin *et. al.*, 2010). The present study is consistent with the other studies like (Zakarea *et. al.*, 2013; Rai *et. al.*, 1995; Al-Abri *et al.*, 2000). In different studies there is high a variation in rate of ACL-Abs. may be due to many causes of repeated abortion in

women and the different methods that employed to determinate antibodies so, these data indicate the necessity of systemic investigation of these antibodies in pregnant women at risk of miscarriage.

## References

- Al- Abri, S.; Vaclavinkova, V. and Richens, ER. (2000). Outcome of pregnancy in patients possessing anticardiolipin antibodies. Al- Abri, S.; Vaclavinkova, V. and Richens, ER. (2000). Outcome of pregnancy in patients possessing anticardiolipin antibodies.
- Arnout, J. and Vermeylen, J. (2003). Current status and implications of autoimmune antiphospholipid antibodies in relation to thrombotic disease. *J. Thromb Haemost.* 1:931-942.
- Beyers, EM.; Comfurius, P. and Dekkers, DW. (1999). Lipid translocation across the plasma membrane of mammalian cells. *Biochim Biophys Acta.* 1439:317-330.
- Branch, DW.; Peaceman, AM.; Druzin, M.; Silver, RK.; El-Sayed, Y.; Silver, RM.; Esplin, MS.; Spinnato, J. and Harger, J. (2000). A multicenter, placebo-controlled pilot study of intravenous immunoglobulin treatment of antiphospholipid syndrome during pregnancy loss study group. *Am. J. Obstet Gynecol.* 182(1 Pt 1):122-7.
- Castro, C. and Gourley, M. (2010). Diagnostic testing and interpretation of tests for autoimmunity. *J. Allergy Clin. Immunol.* 125(2 Suppl. 2): 5238-5247.
- Choudhury, SR. and Knapp, LA. (2001). Human reproductive failure 1: immunological factors. *J. Hum. Reprod. Update.* 7:113-134.
- Clarisa, G.; Mary, D.; Jesse, C.; Amy, H. and Alka, S. (2005). Risk factors for spontaneous abortion in early symptomatic first-trimester pregnancies. 106(5), part 1.
- Cowchock, S. (1996). Prevention of fetal death in the antiphospholipid antibody syndrome. *Lupus.* 5(5): 467-72.
- Daboubi, MK. (2001). Anticardiolipin antibodies in women with recurrent abortion. *East Mediterr Health J.* 7: 95-99.
- Derksen, RH. (2001). How to treat women with antiphospholipid antibodies in pregnancy. *Ann Rheum. Dis.* 60:1-3.
- Heilmann, L.; Von, YG. and Pollow, K. (2003). Antiphospholipid syndrome in obstetrics. *Clin Appl Thromb Hemost.* 9(2):143-50.
- Houtkooper, RH. And Vaz, FM. (2008). Cardiolipin, the heart of mitochondrial metabolic cell. *J. Mol. Life.* 65:2493-2506.
- Jawad, IM.; Mahdi, NK. and Flafil, MS. (2006). Anticardiolipin antibody in women with recurrent spontaneous miscarriage. 27(9).
- Kareem, SR. (2012). First trimester spontaneous abortion: a clinicopathological view. *Diyala J. for Pure Science.* 8 (3):159-174.
- Ong, SG.; Cheng, HM.; Soon, SC.; Goh, E.; Chow, SK. And Yeap, SS. (2002). IgG anti-beta(2) glycoprotein 1 antibodies in Malaysian patients with antiphospholipid syndrome and systemic lupus erythematosus: prevention and clinical correlation. *Clin Rheumatol.* 21(5):382-5.
- Rai, RS.; Clifford, H. and Regan, L. (1995). High prospective fetal loss rate in untreated pregnancies of women with recurrent antiphospholipid antibodies. *Hum. Reprod.* 10(12): 3301-3304.
- Reddel, SW. and Krilis, SA. (1999). Testing for and clinical significance of anticardiolipin antibodies. *Clin. Vaccine Immunol.* November. 6(6): 775-782.

- Risan, FA.(2011). Incidence of anticardiolipin antibodies level in patients with recurrent abortion. Diyala J. of Medicine.1(1):6-10.
- Ruiz-Irastorza,G.; Crowther,M.; Branch,W. and Khmashta,MA.(2010). Antiphospholipid syndrome.Lancet.376:1498.
- Salazar, PM.; Jara ,LJ.; Ramos ,A.; Barile, L.; Machado, G. and Torre, GD.(2002). Longitudinal study of antinuclear and anticardiolipin antibodies in pregnant women with systemic lupus erythematosus and antiphospholipid syndrome. Rheumatol Int.22(4):142-7.
- Speigiorin, LC. Galao,E.; Bagarelli, LB.; Olinari, AH. and Godoy, PD. (2010). Prevalence of anticardiolipin antibodies in pregnancies with history of repeated miscarriages. Open Rheumatol J. 4:28-30.
- Velayulhaprabhu,S. and Archunan, G.(2005). Evaluation of antiphospholipin and antiphosphatidylserine antibodies in women with recurrent abortion. J. Glob. Infec..Dis. 59(8):347-352.
- Zakarea, AY; Nabeel, EW. and Nabaz,EW.(2013).The prevalence of positive serum anticardiolipin antibodies and asymptomatic bacteriuria in women with recurrent abortion.45(issue 1):39-42.