

Research Article

Antiphospholipid Antibodies – a tool for the screening of repeated spontaneous abortionZafar Iqbal¹, Tasneem Zafar¹, Waqas Zafar¹, Tooba Zafar¹, Sana Waqas¹¹Department of Biochemistry, Bakhtawar Amin Medical & Dental College Multan**Abstract**

Received: Nov, 24, 2016
Revised: Nov, 18, 2016
Accepted: Apr, 25, 2017
Online:

To evaluate the biomedical and clinical significance of antiphospholipid antibodies in women with repeated spontaneous abortion. Mohi-u-Din Islamci Medical College & DHQ Hospital, Mirpur, AJ & K, from March to December 2013. 50 subjects with history of three spontaneous abortions in their first trimester of pregnancy were included in this study & fifty women of corresponding age, with one, more alive babies having no record of any first three months spontaneous abortion were taken as controls. Coagulation tests, platelet count, prothrombin time and activated partial thromboplastin time were done by standards methods. Antiphospholipid antibodies were estimated by ELISA method using specific kits. Mean serum antiphospholipid antibodies level was 7.10 ± 3.47 in patients and 6.30 ± 2.02 in controls. The difference in serum level of two groups was significant. Mean platelet count, mean prothrombin time & activated partial thromboplastin time revealed no significant difference between patients and controls. It is concluded that there is a strong association of antiphospholipid antibodies in the patients having recurrent spontaneous abortions so there is a strong need of including this test in the primary screening of such disease in the pregnant women who have history of previous repeated spontaneous abortion.

Keywords: Antiphospholipid antibodies, abortion, prothrombin time, activated partial thromboplastin time**Introduction**

Antiphospholipid antibodies are circulating antibodies to negatively charged phospholipids. They include lupus anticoagulant, anticardiolipin immunoglobulin G (IgG), or IgM antibodies and glycoprotein I antibodies (Nielsen and Christiansen, 2005, Kalra *et al.*, 2002). They may occur alone or in association with lupus. Antiphospholipid antibody syndrome is defined as the presence of at least one antibody in association with arterial or venous thrombosis with or without one or more obstetric complication (unexplained fetal demise after 10 weeks' gestation or severe preeclampsia or fetal growth restriction before 34 week's gestation). Lupus anticoagulant can be screened for with an activated partial prothrombin time (Giasuddin *et al.*, 2010). WHO defines

spontaneous abortion as loss "fetus having weight of 0.5 kg corresponding to the gestational age of twenty to twenty two weeks or less". Recurrent spontaneous miscarriage is defined as the loss of three or more clinically recognized consecutive pregnancies spontaneously before the 24th week of gestation, i.e., loss of three or more pre-viable (< 20 weeks gestation or 500 g) pregnancies. Recurrent abortion occurs in about 1% of all couples. Abnormalities related to recurrent abortion can be identified in approximately half of these instances. If a woman has lost three previous pregnancies without identified cause, she still has at least a 65% chance of carrying a fetus to viability (Meka and Reddy, 2006). Among the multiple factors implicated in the pathogenesis of adverse pregnancy outcome, autoimmune disorders appear to play important role (Dasgupta, 2001). In last two centuries researchers did vigorous work on the association of antiphospholipid antibodies (aPL) and abortion. The Anti- β^2 -

***Corresponding Author:** Zafar Iqbal
Address: Department of Biochemistry, bakhtawar Amin Medical & Dental College Multan
Email address: Prof_bio@ymail.com

glycoprotein I test, which detects antibodies that bind (β^2 -glycoprotein I, a molecule that interacts closely with phospholipids (Salamat *et al.*, 1999, Sikara *et al.*, 2010). The aPL is an antibody (IgG or IgM) that affects the phospholipid dependent coagulation tests by binding to epitopes on the phospholipid portion of prothrombinase. These antibodies have been detected in 16.5% cases of recurrent spontaneous abortion (Tektonidou *et al.*, 2009, Ulander *et al.*, 2007). Women with antiphospholipid syndrome (APS) and antiphospholipid antibodies (aPL) are at high risk for recurrent spontaneous miscarriage. Recent clinical and experimental observations suggest that the pathophysiology of pregnancy failure in patients with APS may involve inflammation at the maternal-fetal interface and disruption of normal trophoblast function and survival, rather than a pro-thrombotic event (Tektonidou *et al.*, 2009, Ulander *et al.*, 2007, Committee on Practice Bulletins—Obstetrics, 2012, Ullah, 2011, Sikara *et al.*, 2010).¹⁴ This study was carried out to analyze antiphospholipid antibodies in women with recurrent spontaneous abortion.

METHODOLOGY

This case control study was conducted at Moh-u-Din Islamic Medical College & DHQ Hospital Mirpur, AJ &K, from March to December 2013. For the selection of patients a total of 100 women were included in the study and Non-probability sampling technique was used. Fifty women were selected for each of two different groups. Group-1: comprises of women with history of three or more first trimester spontaneous abortions and Group – II was control group which include women with one or more live

birth and with no history of any first trimester spontaneous abortion. All fertile women with history of repeated spontaneous abortions were included in this study. Women with no history of abortion were taken as controls. Women having immunological or rheumatic disorders, endocrine disorders, history of thrombosis and those currently on steroid or immunosuppressive therapy were excluded from this study. Patient's whole blood (7.5 ml) was collected, 2.5 ml was transferred in EDTA tube for platelet count, (normal range for platelet count is $150-400 \times 10^9/L$), 2.5 ml in citrated tube for prothrombin time (PT, normal range is 12-16) & Activated partial thromboplastin Time (APTT, normal range 26-37 s), 2.5 ml in gel tube, and serum was separated by centrifugation ($3000g \times 10$ min) and stored at $-80^\circ C$ for analysis of antibodies. Platelet count, PT and APTT were done by routine methods (6). Antiphospholipid Antibodies were estimated by ELISA method using specific kits (Kirkwood and Sterne, 2010).

Data was analyzed using SPSS (Statistical Packages for Social Sciences) version 16.0. Chi-square test and Z-test of proportion were used for comparison of qualitative output response. Mean and SD were calculated with the help of measures of central tendency and measures of dispersion. Statistical significance was taken at $p < 0.05$; (Ulander *et al.*, 2007).

RESULTS

Mean age of group-I was 25.86 ± 0.70 and mean age of group-II, was 24.72 ± 0.73 . There was no significant difference in the mean age of two groups as shown in Table 1.

Table 1: Age Distribution of Patients (n=100)

	Group-I: N=50	Group-II: N=50
Age (years)	25.86± 0.70	24.72± 0.73

Frequency of antiphospholipid antibodies in two groups is shown in table 2. Antiphospholipid antibodies were found in 6 (9.29%) women and absent in 48 (90.71) of Group-I, there were present in 2 (1.47%) women and absent in 48 (98.53) of Group-II.

Table 2: Frequency of Antiphosphatidylserine Antibodies in both Grosup (n=100)

	APA		Total
	Positive	Negative	
Group- I (Cases)	6 (9.29%)	44(89.71)	50
Group-II (control)	2 (1.47%)	48(98.53)	50

Mean serum level of antiphospholipid antibodies is shown in table 3. In Group I mean serum Antiphospholipid antibodies level was 7.10 ± 3 in Group-II was 6.30 ± 2.02. The difference in serum level of two groups was significant.

Table 3: Serum Level of APA in both Groups (n=100)

	Cut off values	Group-I (Cases) N=50	Group-II: (Controls)N=50
Mean APA (RU/ml)	<	7.10±0.42	6.30±0.24

Mean platelet count in two groups is shown in table 4 . In Group-I, the mean platelet count was $255.72 \times 10^3 \pm 79 \times 10^3$ and that in Group-II, it was $260.76 \times 10^3 \pm 71.755 \times 10^3$ the difference was insignificant. Table 4 also shows mean PT and APTT in two groups. In the Group-I the mean PT value was 10.72 ± 1.84 and that in Group-II it was 30.78 ± 2.68 . The difference was insignificant.

DISCUSSION

This study was undertaken to evaluate antiphospholipid antibodies as a casual factor in recurrent spontaneous abortion in women of AJ&K.

Table 4: Mean Platelet count, PT & APTT in both Groups (n=100)

	Cut off Values	Group-I (Cases)	Group-II (Controls)
Mean Platelet count (per/cmm)	150×10^3 - 450×10^3	$258.72 \times 10^3 \pm 79 \times 10^3$	$260.76 \times 10^3 \pm 71.75 \times 10^3$
PT (sec)	12-16	11.72 ± 1.88	11.80 ± 2.68
APTT (Sec)	30-40	29.58 ± 2.61	$29.78 \pm .68$

The possible basic Pathogenesis involved in the spontaneous abortion may be negatively charged phospholipids and a glycoprotein-I is present on the outer surface of apoptotic blebs and so aPLs (Antiphospholipids) are believed to have a similar mechanism to the lupus autoantibodies (Cervera *et al.*, 2002, Meka and Reddy, 2006). Pathogenic aPLs bind to the N-terminal domain of β^2 - glycoprotein I and this interaction is facilitated when the protein is bound to phospholipid on the surface of cells such as endothelial cells, platelets, monocytes and trophoblasts. Between 20 and 30% of patients with SLE possess serum antiphospholipid antibodies. The origin of these antibodies may be similar to that of anti-dsDNA antibodies because monoclonal antiphospholipid antibodies from patients with SLE also show antigen- driven accumulations of somatic mutations. The antigen in this case may be phosphatidylserine on the outer surfaces of blebs derived from apoptotic cells (Nielsen and Christiansen, 2005, Ayyub *et al.*, 2005). Antiphospholipid (aPL.) antibodies may be

present in healthy people or in those with infectious diseases such as syphilis, but with no adverse effects. In patients with SLE, APL antibodies may cause arterial or venous thromboses or miscarriages (Daugas *et al.*, 2002, Ulander *et al.*, 2007). The combination of these clinical problems with the presence of APL antibodies defines the antiphospholipid syndrome (APS). APS may occur either in patients with other autoimmune diseases (particularly SLE), or alone in the absence of other disease (primary APS) (Sikara *et al.*, 2010). Although it was previously thought that APL antibodies exerted their effects almost wholly through promotion of thrombus formation, it is now clear that they may have many other direct effects on platelets, monocytes, endothelial cells, and the trophoblast. The mechanism by which thrombosis is altered is not fully understood, but it has become clear that APL antibodies found in APS often bind to protein antigens associated with phospholipids rather than the phospholipids themselves. The most important of these proteins is β_2 -glycoprotein 1, and a direct test for anti- β_2 -glycoprotein 1 is an alternative diagnostic test for APS (Miyakis *et al.*, 2006, Branch *et al.*, 1997). This alters the functioning of those cells leading to thrombosis and/or miscarriage. In the present study, antiphospholipid antibodies were found in abortion and in 02 cases (1.47%) of control group. Antiphospholipid antibodies as a cause of recurrent abortions have been reported by Lockwood *et al.* with 18% (Branch *et al.*, 1997), and Parrazzani *et al.* with 12% (Lockwood *et al.*, 1986). An antiphospholipid antibody as a cause of

recurrent abortions has also been reported by Branch *et al.* with 16% (Kalra *et al.*, 2002) and Kalra *et al.* with 16.7% (Tariq *et al.*, 2016). An antiphospholipid antibody as a cause of recurrent abortions has also been reported by Giasuddin *et al.* with 10.29% (10.29%) of repeated spontaneous abortion with 37.5% (Ullah, 2011) and Shahida Mohsin *et al.* with 42% (Giasuddin *et al.*, 2010). An antiphospholipid antibody as a cause of recurrent abortions has also been reported by Ayyub *et al.* with 45% (Salamat *et al.*, 1999) and 62% by Saadia Fawad (Vlachoyiannopoulos *et al.*, 2007). In this study, the titre of antiphospholipid antibodies was found to be significantly higher than in controls. This is in agreement with the observations of above workers; Khan in 2004 (Tariq *et al.*, 2016). Shahida Mohsin *et al.* in 2011 (Ullah, 2011) also found the same results. Ayyub *et al.* in 2005 (Ayyub *et al.*, 2005) had also reported the same result and Salamat N *et al.* in 2000 (Salamat *et al.*, 1999) had also reported the same result. Since various previous studies have reported that thromboembolism is associated with antiphospholipid antibodies for causing recurrent abortion (Dasgupta, 2001, Vlachoyiannopoulos *et al.*, 2007, Ulander *et al.*, 2007, Sikara *et al.*, 2010). Coagulation tests and platelet count were included in the present study. In this study, there was no significant difference in platelet count of cases with positive antiphospholipid antibodies as compared to controls. There is no difference in the clotting profile, PT and APTT in patients with antiphospholipid antibodies from those who did not have them. This is in agreement with study of Khan *et al.* 2004 (Tariq *et al.*,

2016), Shahida Mohsin et al in 2011 (Ullah, 2011), Ayyub et al in 2005 (Ayyub *et al.*, 2005) and Salamat Net al in 2000 (Salamat *et al.*, 1999).

CONCLUSION

It is concluded that there is strong association of antiphospholipid antibodies in the patients having repeated spontaneous abortions. Antiphospholipids antibodies has a significant biochemical role in the diagnosis and prevention of repeated spontaneous abortion. It is recommended as a primary screening test for spontaneous & recurrent abortion.

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