Original Article

Levels of Anti-Phospholipid Antibodies in Females with Recurrent Abortions

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Background: Anti-phospholipid syndrome (APL) is an autoimmune thrombophilic condition, which is significantly associated with recurrent miscarriages. The APL antibodies (IgG & IgM) are heterogeneous and found in patients with recurrent abortions. The objective of present study was to evaluate levels of APL antibodies in patients of recurrent abortions.

Study Design:

Place and Duration of Study: The Study wad conducted in the department of Haematology, University of Health Sciences Lahore in collaboration with the Gynae & Obs department of Shalamar hospital Lahore.

Materials and Methods: It was a case control study. Blood samples were collected from 47 patients and 20 controls. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were done on the patients of recurrent abortions and APL antibodies were detected by ELISA method.

Results: In this study overall positivity of APL antibodies was 42%. Mean levels of antiphospholipid IgM and IgG in patients were significantly higher in patients than control ($P< 0.05$). Out of 20 positive patients, 13 were positive for APL IgM and 3 were positive for APL IgG while both (IgM and IgG) were simultaneously found in 4 patients. Most of the abortions occurred in first 58 (78%) and second trimester 16 (22%) of pregnancy. None in third trimester.

Conclusion: The present study demonstrated that elevated levels of APL antibodies (IgG and IgM) indicate a higher-risk pregnancy and unexplained miscarriages can occur in these patients.

Key words: *Anti-phospholipids, Pregnancy, Abortion*

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Introduction

Pregnancy loss is one of the leading problems in women's health issues.[1] Antiphospholipid syndrome (APL) is an autoimmune thrombophilic condition, which is significantly associated with a history of thrombosis, recurrent miscarriages, late fetal death and thrombocytopenia. [2,3] The APL antibodies (IgG & IgM) are heterogeneous in nature having different antigenic specificities.[4,5] Two most commonly studied APL antibodies are lupus anticoagulant (LA) and anticardiolipin which are associated with recurrent abortions.[6,7] More recently, many *in vitro* studies have suggested that antiphospholipid antibodies directly impair trophoblastic function leading to defective implantation and invasion of the uterine deciduas[8,9] . In Pakistan, prevalence of LA with recurrent fetal loss, stroke and thrombosis is significantly high[10] but still, many issues regarding APL in pregnancy remain poorly understood. Therefore, this study was carried out to determine the prevalence and types of APL antibodies (IgG and IgM) in patients presenting with recurrent abortions.

Materials and Methods

It was a case control study conducted in the department of Haematology, University of Health Sciences Lahore in collaboration with the Gynae & Obs department of Shalimar hospital Lahore. This study was approved from the institutional ethics committee.

Out of 245 patients with recurrent abortions, 47 were included according to the selection criteria. These subjects were screened by the obstetricians for other

possible causes like known disorders of uterus, ovary, endocrinal system and chronic illness like diabetes mellitus and hypertension. If no cause was found, then patients were labeled as having idiopathic fetal loss and referred to us for testing. Twenty healthy age matched, non pregnant females with no history of abortions were included as controls. Detailed history of subjects regarding number of pregnancies, abortions and live births was recorded in a proforma.

Prothrombin time (PT) and activated partial thromboplastin time (APTT) were performed by routine manual methods using Global USA kits. Levels of antiphospholipid antibodies were determined by quantitative sandwich enzyme immunoassay using commercially available kit from IBL America which included the antibodies against cardiolipin, phosphatidyl serine, phosphatidyl inositol and phosphatic acid IgG and IgM class.

Measurements of absorbance were taken at wavelength 405 nm on automated ELISA plate reader. Patient's samples having absorbance, more than 10% of cut-off were considered positive, whereas < 10% were taken as negative.

Statistical Analysis

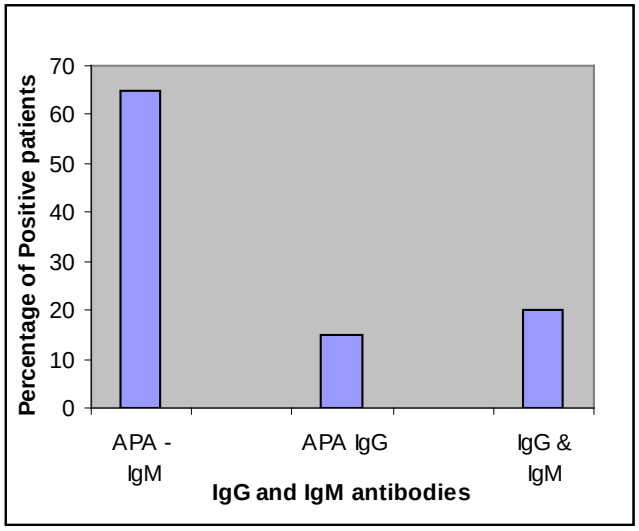
The data was analyzed by using SPSS 16.0. The qualitative variable were expressed as mean ± SD. The categorical variables were reported as frequency and proportion. Two independent sample t test was applied to observe group mean differences. A *p*-value ≤0.05 was considered as statistically significant.

Results

Out of 47 patients, 14 were pregnant and 33 were non pregnant. Mean age of the patients was 28.0±4.4 years. Antiphospholipid antibodies (IgG & IgM) were present in 20 patients; 13 were positive for APL IgM and 3 patients were positive for APL IgG while both APL IgM and IgG were simultaneously found in 4 patients (**Figure-I**). Characteristics of these positive patients are given in **Table -1**.

The mean level of APL was higher in patients (IgM 9.5±10.3 MPL U/ml and IgG 16.7±11.2 GPL U/ml) as compared to controls (IgM 6.4 ± 2.7 MPL U/ml and IgG 2.8 ± 2.7 GPL U/ml) p<0.05. There was no difference of PT and APTT in patients and controls (Table -2)

History of APL positive patients revealed that total 86 pregnancies took place, out of which, 74 terminated prematurely, while remaining 12 pregnancies ended in live births. Most of the abortions happened in first trimester of pregnancy 58 (78%) while others 16 (22%) occurred in second trimester. No abortion occurred in third trimester (**Table - III**).



The relation of APL-IgM and APL-IgG with number of abortions is presented in **Figure-II**.

Figure-I: percentages of positive patients with high titer Ig-M, Ig-G and both (IgG and IgM) antibodies (results has been given for twenty patients).

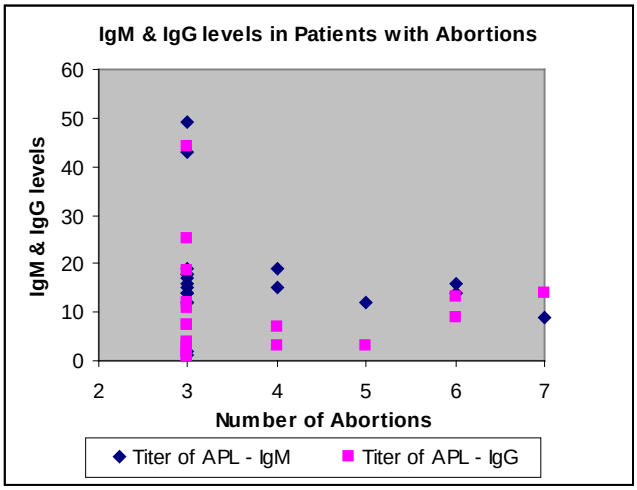


Figure II: High titer (IgG and IgM) APL levels in relation to number of abortions in Positive patients.

Table I: Characteristics of patients with high titer anti Phospholipids antibodies IgG & IgM

Age (Years)	Number of Children	Number of Abortions	Current Status of Pregnancy	aPLA IgM (MPL U/ml)	aPLA IgG (GPL U/ml)
Patients positive for Antiphospholipid antibodies – IgM (13 patients)					
29	1	5	No	12	3
35	2	3	No	16	4
20	0	3	yes	14	2
27	0	3	No	14	0.7
24	0	6	Yes	14	9
35	2	4	No	15	3
27	0	3	No	19	1.4
29	0	3	Yes	17	2.6
35	3	3	No	18	1.3
30	1	3	No	43	3.6
25	0	3	No	18	7.5
24	0	4	No	19	7
32	0	3	No	49	7.2
Patients positive for Antiphospholipid antibodies – IgG (03 patients)					
29	1	7	yes	9	14
32	0	3	No	2	18.5
29	0	3	No	1	12
Patients positive for Antiphospholipid antibodies - IgM & IgG (04 patients)					
22	0	3	No	15	11
25	0	6	No	16	13
30	2	3	No	12	44
30	0	3	No	12	25

APL – Antiphospholipid. Data is given for 20 APL antibodies positive patients.

Table-II: Levels of PT, APTT and Anti-phosphlipid IgM and IgG in patients and controls

Parameters	Patients	Controls	*p*-value
	(Mean±SD)	(Mean±SD)	
APL IgM (MPL U/ml)	9.5±10.3	6.4 ± 2.7	0.005
APL IgG (GPL U/ml)	16.7±11.2	2.8 ± 2.7	0.015
PT	14.3 ± 0.7	14.4±0.7	0.83
APTT	34.5 ± 0.9	34.5±0.9	0.686

Data is given for 47 patients and 20 controls

Table III: Pregnancies / Abortions in APL Positive and Negative Patients

Parameters	Positive for APL subjects (n=20)	Negative for APL subjects (n=27)
Present status of Pregnancy	4	10
Total Pregnancies	86	65
Total Abortions	74	48
Abortions in 1st trimester	58	37
Abortions in 2nd trimester	16	11
Abortions in 3rd trimester	Nil	Nil
Alive issues	12	17

APL: Antiphospholipid antibodies

Discussion

APL antibodies are considered to be a significant predictor of fetal loss.[1] Women who are positive for APL antibodies have increased risk of repeated early miscarriages and second or third trimester fetal deaths because sera of these women contain antibodies reactive against trophoblastic cells, which are involved in the establishment of uteroplacental vasculature and maintenance of placental blood fluidity. These antibodies may exert a direct pathogenic effect by interfering with haemostatic process that takes place on the phospholipids membranes of cells such as platelets or endothelium. It is also suggested that APL auto-antibodies may cause intravascular coagulation leading to recurrent abortions.[11,12]

In the present study APL antibodies were found in 42% patients which is supported by other studies[6, 13, 10,14]. It was found that the percentages of positivity for APL IgM, IgG, and both (IgG and IgM) were 27.0 %, 8.0 % and 7.0 % respectively. While in other studies, the positivity of APL IgM, IgG, and both (IgG and IgM) were (8%, 7% and 4%) [13] (13 %, 5 % and 3 %) [14], respectively. It shows that IgM positivity of our patients (27%) was higher as compare to other studies [13, 14.] This may be due to transient and non specific nature of IgM antibodies, because these antibodies also increase secondarily to drug exposure and infections. Antiphospholipid IgG antibodies were more specific and commonly associated with thrombosis, thrombocytopenia and recurrent fetal loss .[11]

It has also been found that APL antibodies were significantly high in patients with abortions as compared to controls as has been reported in previous studies. [5,11,14]

In our study 20 patients were found to be positive for antiphospholipid antibodies. They had wide range of abortions (3-7). Proportion of recurrent abortions was significantly high ($P<0.05$) amongst this group of patients. Total 86 pregnancies took place in these patients. Out of 86 pregnancies, 74 terminated prematurely. Remaining 12 pregnancies ended in live births. Similar findings were also observed by Indu kaul *et al* , they reported that total 33 pregnancies occurred, out of which 78% terminated prematurely, 18 (54%) losses occurred in first trimester and 4 (12%) each in the second and third trimester.[14] In our study we found all abortions occurred in first (78%) and initial phase of second trimester (22%) of pregnancy, no abortion occurred in third trimester.

PT and APTT of our patients were within normal range as it was seen by Salamat *et al* [5]. All controls were negative for antiphospholipid antibodies as found by Kaul et al and Daboubi. [11]

Conclusion

The present study demonstrated that elevated levels of APL antibodies (IgG and IgM) indicate a higher-risk pregnancy and unexplained miscarriages can occur in these patients.

On the basis of our results, it can be recommended that women with recurrent unexplained miscarriages should always be screened for Anti phospholipids antibodies.

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