

Original Research Article

To evaluate the prevalence of anticardiolipin antibodies among women with recurrent abortions and to determine any relation between anticardiolipin antibodies and number of abortions and their gestational age of abortions

Dr. Sumitra Yadav¹ (Professor), Dr. Sapna Chaurasia² (Assistant Professor) & Dr. Shaniya Mirza³ (3rd Year PG Resident)

Dept. of Obstetrics and Gynaecology, Mahatma Gandhi Memorial Medical College, Indore, M.P.^{1,2&3}

Corresponding Author: Dr. Shaniya Mirza

Abstract:

Background & Method: The aim of present study is to evaluate the prevalence of anticardiolipin antibodies among women with recurrent abortions and to determine any relation between anticardiolipin antibodies and number of abortions and their gestational age of abortions. An indirect Non-competitive enzyme immunoassay for the semi quantitative and qualitative determination of cardiolipin IgG antibodies is performed. The wells of the microtitre plate are coated with cardiolipin antigen. Antibodies specific for cardiolipin present in the patient's sample bind to the antigen. The second step, the antigen antibody complex reacts with an enzyme labelled second antibody (Enzyme Conjugate) which leads to the formation of an enzyme labelled antigen antibody sandwich complex. The enzyme label converts added substrate from a colored solution.

Result: The mean anti cardiolipin IgG level was more among cases with 10.12+/-71 units compared to 6.12+/-4.96 among controls and it was statistically significant ($p < 0.001$). But there were no significant difference exists between cases and controls in terms of IgM antibodies. In the present study if the IgG anti cardiolipin antibody level of >10 then it was considered abnormal. It was evident from the above table that the prevalence of abnormal IgG level was significantly higher in cases (32.9%) compared to controls (8.6%).

Conclusion: IgG anti cardiolipin antibody level of >10 was considered abnormal. In this study the adverse outcome of the pregnant women was assessed in terms of intra uterine death (IUD), intrauterine growth retardation (IUGR), Small for gestational age (SGA), Preterm delivery, Abruptio and early onset pregnancy induced hypertension (PIH). It is evident that, if the women had abnormal IgG level, then the incidence of adverse outcome was more compared to those with normal IgG level and it was statistically significant. If we check the individual adverse events, the incidence of IUD, IUGR, Abruptio and Early onset PIH were significantly more among those with abnormal IgG anticardiolipin antibodies.

Keywords: anticardiolipin, antibodies, abortions & gestational age.

Study Designed:

1. INTRODUCTION

The science of immunology is one such breath taking field and the ability of human being to discriminate between self and non-self-antigens is phenomenal[1]. The recognition and the

response to foreign antigens, an ability perhaps most highly developed in mammalian species is one of the most intensively studied topics in science and medicine today. Pregnancy is an unique immunological stage where a natural homeostasis exists between antigenically different tissues.

One of the Medawars initial hypothesis, on the maintenance of the foetal allograft was that pregnancy was associated with suppression of maternal immune response thereby allowing fetal survival. The pregnant host however must also maintain immune competence against both pathogenic and neoplastic invasion during the gestation to ensure host survival[2].

Autoimmunity is one deviation from this normal pattern, where antibodies are directed against one's own antigens[3]. One of the fundamental questions in the study of any autoimmune disease concerns the role of auto antibodies in the pathogenesis of the disorder. In certain autoimmune conditions like Grave's disease and Myasthenia gravis the evidence that they are directly involved in the pathogenesis is strong.

Recurrent pregnancy loss is defined as 2 or more unexplained first trimester loss and 1 or more unexplained second trimester loss. Accepted etiologies for RPL include parental chromosomal abnormalities, untreated hypothyroidism, uncontrolled diabetes mellitus, uterine abnormalities and anti-phospholipid antibody syndrome[4].

Much recently interest has been focused over the Antiphospholipid Antibody syndrome. Antiphospholipids were first linked to pregnancy loss more than 30 years ago and the condition known as antiphospholipid syndrome is perhaps the most convincing immune disturbance other than anti erythrocyte and antiplatelet alloimmune disorders[5]. Specific criteria for antiphospholipid syndrome have been delineated and the anticardiolipin assay has been standardized and authorities have agreed on laboratory criteria to define lupus anticoagulant.

2. MATERIAL & METHOD

The present study was conducted in department of Obstetrics and Gynaecology, MGM Medical College and MYH hospital, Indore during a period of two years from date of approval.

- A consecutive series of 70 women with history of recurrent abortions and 70 women with no previous pregnancy loss were taken.
- A detailed history with physical and gynaecological examination was done.
- In both groups, Group A and Group B various blood samples were collected and serum was separated and result was estimated by Indirect ELISA, Cardiolipin diluted in ethanol with or without chloroform coated into standard non irradiated 96, well polygrene plate by vacuum or overnight during at 40C*. Immunoglobulin isotype specific second antibody was used to assay ACL of IgG, IgM. On the basis of Titre results prevalence of antibody was determined.

An indirect Non-competitive enzyme immunoassay for the semi quantitative and qualitative determination of cardiolipin IgG antibodies is performed. The wells of the microtitre plate are coated with cardiolipin antigen. Antibodies specific for cardiolipin present in the patient's sample bind to the antigen. The second step, the antigen antibody complex reacts with an enzyme labelled second antibody (Enzyme Conjugate) which leads to the formation of an enzyme labelled antigen antibody sandwich complex. The enzyme label converts added substrate from a colored solution.

INCLUSION CRITERIA**GROUP A**

1. Age-group 18-45 years
2. Women with history of 2 or more spontaneous abortion
3. Patient who gave informed written consent

GROUP B

1. Age group 18-45 years
2. At least 1 live children with no history of any previous pregnancy
3. loss.
4. Patients who gave informed written consent.

EXCLUSION CRITERIA

1. Age less than 18 and more than 45 years of age
2. VDRL Positive females
3. Women with infections such as hepatitis Malaria, HIV
4. Coagulation Disorders
5. Uterine anomaly
6. Induced abortions
7. Women who did not give consent
8. FOR ANTICARDIOLIPIN ANTIBODIES (IgG/IgM)
9. Preparatory maturation during specimen collection
10. No fasting required
11. No other special preparation required

4. RESULTS**Table 1: COMPARISON AMONG CASES AND CONTROLS WITH RESPECT TO GRAVIDITY, LIVE BIRTHS AND ABORTIONS**

Variables	CASES		ABORTIONS		Chisquare test
	N	%	N	%	
2	0	0.0%	11	15.7%	0.003
3	21	30.0%	25	35.7%	
4	30	42.9%	22	31.4%	0.003
5	19	27.1%	12	17.1%	
0	32	45.7%	0	0.0%	

1	32	45.7%	11	15.7%	
Live					
2	6	8.6%	25	35.7%	<0.001
birth					
3	0	0.0%	22	31.4%	
4	0	0.0%	12	17.1%	
2	52	74.3%	0	0.0%	
Abortion					Na
3	18	25.7%	0	0.0%	

The number of gravidities were more in cases compared to controls and live birth was more in controls compared to cases because the cases are those with history of abortions, hence the number of live births were less and gravidity was more.

Table 2: Gestational week at the time of previous abortion among cases

	Mean	SD	Minimum	Maximum
First abortion	13.4	3.5	6.0	18.0
Second abortion	12.4	3.5	6.0	19.0
Third abortion	12.7	3.2	7.0	18.0

In the present study, the gestational week at which abortions happened among cases were enquired and found that the average gestational week at which first abortion happened was 13.4+/-3.5 weeks, second abortion at 12.4+/-3.5 weeks and third abortion at 12.7+/-3.2 weeks.

Table 3: Duration of post abortion to current pregnancy

	Mean	SD	Minimum	Maximum
First abortion	6.2	2.5	2.0	11.0
Second abortion	4.2	2.4	1.0	9.0
Third abortion	2.7	1.9	1.0	7.0

The average duration between the current pregnancy and past abortion was found to be 6.2+/-2.5 years for first abortion, 4.2+/-2.4 years for second abortion and 2.7+/-1.9 years for third abortion.

Table 4: MEAN ANTICARDIOLIPIN IgG &IgM ANTIBODY LEVELS AMONG CASES AND CONTROLS.

Auto antibody	CASES		CONTROLS		Unpaired t test
	Mean	SD	Mean	SD	p value
IgG	10.12	7.10	6.12	4.96	<0.001
IgM	.53	.87	.40	.22	0.217

The mean anti cardiolipin IgG level was more among cases with 10.12+/-71 units compared to 6.12+/-4.96 among controls and it was statistically significant (p<0.001). But there were no significant difference exists between cases and controls in terms of IgM antibodies.

Table 5: COMPARISON BETWEEN CASES AND CONTROLS ON THE BASIS OF ABNORMAL AND NORMAL IgG ANTICARDIOLIPIN ANTIBODY LEVELS.

IgG	CASES		CONTROLS		Chi square test
	N	%	N	%	p value
Abnormal	23	32.9%	6	8.6%	<0.001
Normal	47	67.1%	64	91.4%	
Total	70	100.0%	70	100.0%	

In the present study if the IgG anti cardiolipin antibody level of >10 then it was considered abnormal. It was evident from the above table that the prevalence of abnormal IgG level was significantly higher in cases (32.9%) compared to controls (8.6%).

5. DISCUSSION

A prospective comparative study was conducted among 140 pregnant women, in which 70 women had history of abortions in the past (cases) and 70 did not have history of abortions (controls). The mean age among cases were 30.1 years and among controls 30.3. In DERKSON et al.'s study of anti-cardiolipin antibodies in recurrent pregnancy loss, the mean age was 32 yrs. In Friedman's study mean age was 32 yrs. The increase in incidence in younger patients in this study is probably due to the early marriage in our society[6].

In the present study if the igG anti cardiolipin antibody level of >10 then it was considered abnormal[7]. It was evident from the above table that the prevalence of abnormal igG level was significantly higher in cases (32.9%) compared to controls (8.6%). The mean anti cardiolipin igG level was more among cases with 10.12 units compared to 6.12 units among controls and it was statistically significant (p<0.001). But there were no significant difference exists between cases and controls in terms of igM antibodies[8].

The number of gravidities were more in cases compared to controls and live birth was more in controls compared to cases because the cases are those with history of abortions, hence the number of live births were less and gravidity was more.

The average gestational week at which first abortion happened was 13.4 weeks, second abortion at 12.4 weeks and third abortion at 12.7 \pm 3.2 weeks. 65.7% of women had an interval of \leq 5 years from the second abortion to current pregnancy and 94.4% of women had an interval of \leq 5 years from the third abortion to current pregnancy. In the present study the adverse outcome of the pregnant women was assessed in terms of intra uterine death (IUD), intrauterine growth retardation (IUGR), Small for gestational age (SGA), Preterm delivery, Abruption and early onset pregnancy induced hypertension (PIH).

If the women had abnormal IgG level, then the incidence of adverse was more (58.6%) compared to those with normal IgG level (4.5%) and it was statistically significant ($p < 0.001$). RAI et al. Studied women with recurrent miscarriages and showed a prevalence of 5.5% of anticardiolipin antibody positive in them[9]. In his study showed a prevalence of 15.8% of anticardiolipin antibodies in women with intrauterine fetal deaths. In this study suggested that the prevalence of anticardiolipin antibody is very low in first trimester losses and is not very significant.³³ But MACLEAN and colleagues in their study proved that there was a prevalence of 8.2% in first trimester losses.

6. CONCLUSION

In the present study, IgG anti cardiolipin antibody level of >10 was considered abnormal. In this study the adverse outcome of the pregnant women was assessed in terms of intra uterine death (IUD), intrauterine growth retardation (IUGR), Small for gestational age (SGA), Preterm delivery, Abruption and early onset pregnancy induced hypertension (PIH). It is evident that, if the women had abnormal IgG level, then the incidence of adverse outcome was more compared to those with normal IgG level and it was statistically significant. If we check the individual adverse events, the incidence of IUD, IUGR, Abruption and Early onset PIH were significantly more among those with abnormal IgG anticardiolipin antibodies.

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