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Original Research Article

The Association of Antiphospholipid Antibodiesin Women with Bad Obstetric History

Priti Kumari[1], Sudha Kumari[2], Shashikar[3]

[1]Senior Resident, Department of Obstetrics and Gynaecology, JLNMCH, Bhagalpur
[2]Assistant Prof. Department of Obstetrics and Gynaecology, JLNMCH, Bhagalpur
[3]Department of Radiodiagnosis, Bhagalpur

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Corresponding author: Dr. Sudha Kumari
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Abstract

Background: APL antibodies are the most important autoimmune cause of recurrent fetal loss. These pregnancies can be saved if diagnosed and treated adequately. This can be doneby routine screening for the APLA in a pregnant woman with a bad obstetric history (BOH) and unexplained fetal loss.

Material and methods: The present study is a prospective study carried out in department of Obstetrics and Gynecology in, JLNMCH, Bhagalpur. It was conducted over a period of One and half years. 6 among the women with h/o previous >2 abortions, intrauterine deaths, early neonatal deaths, still births, patients with h/o early onset pre-eclampsia, intrauterine growth restrictions. Patients underwent screening for APLA i.e Lupus Anticoagulant (LA), Anticardiolipin (ACL) and Anti Beta$_2$ glycoprotein(β2-GP1) 1 IgG/IgM. If tested positive, patient underwent a repeat test 12 weeks later.

Conclusion: It is a proved fact that APLA interfere with the normal development of the uteroplacental circulation to cause early and late neonatal pregnancy loss. Hence screening for APLA in patients with BOH will help in identifying the cause ofrecurrent fetal loss and improve the obstetric outcome.

Keywords: BOH, Anticougulant, Anticardiolipin, IgG, IgM, fetal loss.

Introduction

Death of the fetus in utero or at birth has always been mental and social trauma for themother and an unfortunate experience to the obstetrician in their clinical practice. Thus, becoming a challenge when 60% of the recurrent spontaneous abortions are unexplained. About 20% of the women in this world would have atleast one fetal loss while recurrent spontaneous abortions are often seen in 5% of fertile couples. These can be due to various causes like anatomical defect of uterus, chromosomal abnormalities, endocrinal imbalances, subclinical infections and immunological disturbances. The immune factors are classified as autoimmune and alloimmune. One of the most important immunological causes for the fetal loss is antiphospholipid (APL) antibodies. These APL antibodies are autoimmune factors. The association of women with bad

obstetric history (BOH) is significant. BOH implies the pregnancies with more than 2 recurrent pregnancy loss, intrauterine fetal deaths, intrauterine growth retardations, oligohydramnios, early onset preeclampsia. These women have unfavourable pregnancy outcomes. Other placenta mediated pregnancy complication has also been associated with presence of antiphosholipid antibodies. The main types of antiphosholipid antibodies are lupus anticoagulant, anticardiolipin and anti-β2-glycoprotein 1. Fetal morbidity and mortality have also been found to be higher in mother with antiphosholipid antibodies positivity. Hence all these complications are statistically significant when compared to those with antiphosholipid antibodies negative mothers. Although APL antibodies are seen in 2-5% of general population, it has been found to be the most important acquired cause for recurrent fetal loss. It is a proved fact that the antiphospholipid antibodies interfere with the normal development of the uteroplacental circulation to cause both early and late neonatal pregnancy loss. Hence many pregnancies can be saved if diagnosed and treated adequately. This can be done by routine screening for the antiphospholipid antibodies in a pregnant woman with BOH and unexplained fetal loss.

Objectives

To investigate the association of antiphospholipid antibodies in women with bad obstetric history and thus evaluating the usefulness of routine screening of serum antiphospholipid antibodies in patients with h/o unexplained fetal wastage and bad obstetric history for better outcome.

Review of Literature

Hemostasis is the result of highly regulated process that maintain the blood in the fliud state in normal vessels and also helps in the formation of hemoststic clot at the site of vascular injury. The pathological counterpart of this hemostasis is thrombosis which involves the formation of thrombus in the intact vessels. Normal hemostasis [1] Normally there is a brief arterial vasoconstriction after the injury which ismediatedby reflex neurogenic mechanism and augmented by local secretion of endothelin which is a potent vasoconstrictor. The injured endothelium then exposes highly thrombogenic subendothelial extracellular matrix (ECM), which facilitates the platelet adherence and activation. The activated platelets then acquire dramatic shape and release secretory granules, which facilitates the additional platelet aggregation and hemostatic plug formation. This process is called primary hemostasis. Tissue factor, also known as factor III and thromboplastin is a membrane bound procoagulant glycoprotein synthesized by the endothelial cells. It acts along with factor VII as a major initiator of coagulation cascade inside the body, resulting in thrombin formation. Thrombin then breaks the circulating soluble fibrinogen into fibrin monomer which further polymerize to form insoluble gel, thus forming fibrin meshwork. The above mechanism further activates the platelets and its recruitment. This process is called secondary hemostasis which consolidates the initial platelet plug. Normal blood flow is laminar such that the platelets and other cells flow centrally, away from the endothelium separated by a layer of plasma. The endothelial integrity is important factor. Any injury to the endothelium can change the local blood flow and can affect coagulability. Coagulation cascade play a vital role in maintaining the melieau of hemostasis. It is a complex process and essentially is a multiplying series of enzymatic conversions, each step proteolytically cleaves an inactivated proenzyme into activated enzyme resuting in the formation

of thrombin. This thrombin in turn converts the fibrinogen to polymerized insoluble fibrin which encases the platelets and other circulating cells in the formation of secondary hemostatic plug. Each reaction in this pathway results in the complex which is composed of an enzyme (activated coagulation factor), a substrate (proenzyme form of coagulation factor) and a cofactor (reaction accelerator). These components are together assembled on phospholipid surface and held together by calcium ions. Three or more consecutive pregnancy losses at <20 weeks or with a fetal weight <500 grams. [2]

The American society for reproductive medicine (2008) proposed that recurrent pregnancy loss is defined as two or more failed clinical pregnancies confirmed by either sonographic or histopathological examination. [3] This change in the definition is due to lack of statistic difference in pregnancy outcome between women who had previous 2 miscarriages and the women with 3 miscarriages. More over the patients are naturally anxious about their future pregnancies and hence there is a need to carry out investigations in these patients. Bad obstetric history (BOH) implies previous unfavorable fetal outcome in terms of two or more consecutive spontaneous abortions, early neonatal deaths, stillbirths, intrauterine fetal deaths, intrauterine growth retardation. [4] Preeclampsia [**5]** Increase of blood pressure >140/90 after 20 weeks of gestation in previously normotensive patient associated with proteinuria. APLA syndrome also known as Hughes syndrome is a intriguing autoimmune syndrome, recognized to have a major contribution in the recurrent pregnancy loss (RPL) since past 2 decades. [6] It is discovered as one of the treatable cause and hence diagnosing APLA in high risk individuals is essential for better obstetric outcome. APLA are group of heterogenous antibodies directed against negatively charged phospholipids (PL) or PL binding proteins and thus causes placental thrombosis, infarction and impair trophoblastic function by mechanism unrelated to the thrombosis. [7] They were first detectd in 1906 in the patients who had false positive test for syphilis. [8] It was only in 1952 that Country and Hartman described them in SLE paients. PL are the main lipid constituents of the cell and organelle. They are present on almost every cell surface. The antibodies to the anionic PL haveaffinity for platelets and vascular endothelium. Thus this event causes arterial and venous thrombosis like deep venous thrombosis, pulmonary embolism, transient ischemic attack, stroke, chorea, lividoreticularis, thrombocytopenia etc apart from the RPL. [9] APLA was originally discoved in systemic lupus erythematosis (SLE) patients, and this association has 40% of thrombosis than those without SLE association. [10] APLA are seen in 1-5% of general population [11] and more commonly in elderly. These antibodies are trigerred by infection, chronic inflammation, malignancy, stress and drugs. It is seen 15-20% of high risk population. [12] The basic line of action of APLA is at feto-maternal interface. Physiological changein pregnancy wherein the trophoblasts invade the spiral arterioles and destroys the intima replacing the endothelial lining. This converts the feto-materanl interface into a low resistance pool of blood which is vital for normal pregnancy. SN Rai R, Cohen H did a comparative study wherein the aspirin along with heparin had successful pregnancies (71-80%) as compared to aspirin alone (42-44%) [13]. The steroids can also be associated with other obstetric complications like premature rupture of membranes, preterm lobour, diabetes, hypertension, osteoporosis and psychosis. [14]

Material and Methods

Prospective observational study, carried

out in hospital patients with history of (h/o) bad obstetric history like the pregnancies with more than 2 recurrent pregnancy loss, intrauterine fetal deaths, intrauterine growth retardations, oligohydramnios, early onset preeclampsia, attending antenatal clinic or in-patient in Department of Obstetrics and Gynaecology of Jawahar Lal Nehru Medical College and Hospital, Bhagalpur, Bihar. Study duration of One and half years.

Inclusion criteria

Women with h/o previous 2 or more abortions, H/o intrauterine deaths, early neonatal deaths, still births, Patients with h/o early onset pre-eclampsia, Intrauterine growth restrictions, Unexplained oligohydramnios

Exclusion criteria

H/o overt diabetes mellitus, gestational diabetes mellitus, congenital anomalies

After thorough history and clinical examination, routine blood investigations were carried out. Patients were counselled about the benefits APLA test and its implications for the pregnancy outcome. Patients blood were then collected in two separate bulbs- one in plain bulb (2ml of blood) which is used to detect aCL and β2GP1 antibody by ELISA method and other in citrate bulb (containing 0.2ml of tri sodium citrate 3.2% +1.8ml of freshly collected blood) which is used to detect LA. The collected sample (both plain and citrate bulb) was then centrifuged at the rate of 1500g or 15min to obtain platelet poor plasma. Highly purified cardiolipin is bound to microwells saturated with withβ_2 GP1. Antibodies against these antigens, if present in a diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG and IgM immunologically detect the bound patient antibodies forming a conjugate/ antibody/ antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour.

Anticardiolipin antibodies

(Assay-AUTOBIND AnticardiolipinIgG/IgM)

Normal	<10GPL/ml
Elevated	>10GPL/ml

Results

Table 1: Age distribution APLA negative and positive test.

APLA	Age		Total
	<25	**>25**	**Total**
Negative	20	27	47
	76.9%	87.1%	82.5%
Positive	6	4	10
	23.1%	12.9%	17.5%
Fisher's exact test	p-value = 0.49(NS)		

*p<0.05 statistically significant, p>0.05 Non significant, NS

57 total cases 47 were found to be APLA negative and 10 cases were APLA positive (17.5%). Fig 1a and 1b shows the total 10 APLA positive cases in which 6 cases of APLA positive were below 25yr age group and 4 cases wereabove 25yr age group.

Table 2: Baseline APLA positive and repeated tests after 12 weeks

APLA	Baseline	12 weeks	
		Positive	Negative
Negative	43	0	43
		0.0%	100%
Positive	14	10	4
		71.4%	28.6%
Total	57	10	47
		17.5%	82.5%
Mcnemar test		p-value = 0.13(NS)	

*p<0.05 statistically significant, p>0.05 Nonsignificant, NS

APLA was positive in 14 cases when tested for the first time. When these positive results subjected for the repeat test after 12 weeks, 4 test were negative (7%). Hence in our study 10 out of 57 cases are APLA positive (17.5%) APLA antibodies detected in the study. The positive samples are repeated 12 weeks later in which 3 samples were LA negative and 2 samples each for IgG and IgM of β2-Glycoprotein 1 were negative. There was total 2 Anticardiolipin antibodies IgM (16.6%), 8 samples were Lupus anticoagulant positive (66.6%) and 1 positive for β2-Glycoprotein 1 antibodies IgG and IgM each (8.3%). shows among 10 APLA positive cases, 8 were started on LMWH. There is statistically significance among the APLA positive patients started on Heparin as compared to APLA negative the distribution of APLA antibodies wherein there was no sample positive for Anti cardiolipin IgG. 8 samples were positive for Lupus anticoagulant (p<0.001) and 2 samples positive for Anti cardiolipinIgM (p=0.03). the total number of cases conceived during the study and their mode of delivery. There was total 12 (33.3%) cases who underwent LSCS, 18 (50%) patients underwent preterm vaginal deliveries, and 6 (16.7%) cases underwent full term vaginal deliveries. Out of 12 patients undergoing LSCS 4 cases were APLA positive and 8 were APLA negative. 2 cases undergoing full term vaginal deliveries were APLA positive. 2 cases of APLA positive cases underwent preterm vaginal deliveries.

Table 3: Neonatal outcome in the study

Neonatal outcome	APLA POSITIVE	APLA NEGATIVE	TOTAL
NICU admission	2(22.8%)	7(77.8%)	9
Mother side	5(38.5%)	8(61.5%)	13
IUD	9	3	12
Vascular thrombotic complications	2	1	3
Association with rheumatoid arthritis	2	1	3

the past complications among the patient in the study. Majority (35) had history of more than 2 miscarriages and 4 patients with second trimester loss. Pre-eclampsia and associated complications were seen in 6 patients. There were 12 cases of intrauterine deaths with unknown etiology and 1 case of intrauterine growth restriction. Vascular thrombotic complications were present in 3 cases and 1 case was APLA positive. Rheumatoid arthritis was seen in 3 of the total cases

and 1 patient was found positive for APLA. all the patients in the study group had past or present complications at the time of study. Among 57 cases 45 patients had past complication and 26 had present complications. There were 15 cases in the study who had past complications and presented with complication during the current pregnancy. Hence our study was conducted in high risk patients with complications which are more often seen in patients with APLA.

Discussion

The overall positivity of APLA syndrome in high risk patients in our institute is 17.54% as shown in fig 1a which is significantly less as compared to the study which was conducted in 2007 by Sonal Vor et al from KEM Hospital Parel Mumbai, where 430 women with unexplained fetal loss were screened for the presence of antiphospholipid antibodies. They also studied 100 normal healthy women who had at least one healthy child and did not have any miscarriage or other obstetric complications. They found that the overall APLA positivity was 42.6% in the patient group and 6% in normal healthy individuals [15]. the age distribution of APLA positive antibodies. There were 6 (23.1%) APLA positive cases below the age of 25 year and 4(12.9%) cases above 25 years. Another study by Saha SP et,al found that the prevelance of antiphospholipid antibody ranged between 10- 46.87% in women with BOH and compared with 8.49% in women with normal pregnancies without complication [16]. Whereas Singh &Sidhu studied various factors responsible for the pregnancies with BOH and found that the prevalence of antiphospholipid antibody in test group of high risk pregnancies was 10.13% which islower than our study. [17] The study was conducted in 57 patients of high-risk pregnancies with history of recurrent pregnancy loss, early onset pre-eclampsia, eclampsia, unexplained intrauterine deaths, intrauterine growth restriction. Out of 57 cases 17 cases were found to be APLA positive which included Anti cardiolipinIgG/IgM, Anti β2 glycoprotein-I IgG/IgM and Lupus anticoagulant. In the current study the overall Anticardiplipin antibodies are 3.5% which was associated with complicated pregnancies as shown in table 3 and fig 3. These antibodies cause the pregnancy loss by defective implantation and subsequently causing defective placentation. A study conducted by Nadia Mudher Al-Hilli and Mohammad Al-Mosawi concluded that out of 117 patients with BOH, 11.96% of the cases was positive for anticardiolipin antibodies. They divided the patients by age group and type of fetal loss. The study also concluded that 2 or 3 recurrent miscarriages was the most common type of fetal loss. [4] Velayuthaprabhu, Archunan studied 155 patients in a sequential study with history of recurrent miscarriages and found that 40% of them were positive for anticardiolipin antibodies. [18] Studies also suggests that β_2 glycoprotein-I dependent Anticardiolipin antibodies are strongly associated with recurrent pregnancy loss as compared to β2 glycoprotein-1 independent Anticardiolipin antibodies. β2 glycoprotein-1 is a phospholipid binding protein and is one of the most important antigenic targets for APLA. Studies have shown that β2 glycoprotein-1 can adhere to trophoblasts, which in turn affect the trophoblastic differentiation by inhibiting gonadotrophin release and matrigel invasiveness, which is explained by the process involved in the defective placentation resulting in miscarriages. [19] This was compared to the similar result in the study by Hidetoet. al. where he found that the APLA antibodies can increase the risk of early onset pre eclampsia [20]. In literature, the estimated prevalence of APLA antibodies in rheumatoid arthritis patients varies from 4% to 49%, with the average prevalence of 28%, based on the

data collected from several other studies. [21] Radosław et.al studied in 97 patients with rheumatoid arthritis and found the prevalence of APLA antibodies in 27 patients (27.8%) among which anticardiolipin antibodies was seen in 20 patients (20.6%), β2 –Glycoprotein-1 in 12 patients (12.4%), and Lupus**,** anticoagulant in 1 patient (1%). [22] cross diagram of the past and present complications in which all the patients in the study group had complications at the time of study. [23] Among 57 cases 45 patients had past complication and 26 had present complications. There were 15 cases in the study who had past complications and presented with complication the the current pregnancy. Hence our study was conducted in high risk patients with complications which are more often seen in patients with APLA.

Conclusion

The association of APLA antibodies in high risk pregnancies with history of recurrent pregnancy loss, early onset pre-eclampsia and its complications, unexplained intrautertine death and growth restriction was found to be 17.5% in our study. Although the incidence of APLA antibodies in general population is 1-5%, It is a proved fact that the antiphospholipid antibodies interfere with the normal development of the uteroplacental circulation to cause both early and late pregnancy loss.

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