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Research Article

Antiphospholipid antibodies in pregnant women with preeclampsia: a case control study

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Abstract

Preeclampsia is a major cause of maternal morbidity and mortality, complicating 3-14% of all pregnancies. Pregnancy complicated by antiphospholipid antibodies and preeclampsia in pregnancy share several common characteristics, which suggest that the association between the two conditions is more than coincidental. To assess the level of antiphospholipid antibodies in normal pregnancy and in pregnancy complicated by preeclampsia a case control prospective study carried out in Department of Obstetrics and Gynecology/Baghdad Teaching Hospital/ Medical City/ from January 2012 to January 2013. Seventy-two pregnant women were enrolled into this study, 48 with preeclampsia (mild and severe), and 24 women without hypertension as a control group. Their age ranges from (16-42 years) and gestational age between (24-40 weeks). Antiphospholipid antibodies APLAB (IgG and IgM) and anticardiolipin antibodies ACLAB (IgG and IgM) levels were measured by ELISA (enzyme. linked Immuno-Sorbent Assay) in all participates. The study showed elevation of ACLAB IgM in preeclampsia group with mean ±SD of (21.93±16.12), (8.22±1.75) for severe and mild preeclampsia respectively while in the control group it was (6.62± 2.8). This was highly significant in severe preeclampsia when compared to mild and control group. We conclude that there is significantly elevated level of ACLAB IgM in severe preeclampsia when compared to normal pregnancy.

Key words: Preeclampsia; Antiphospholipid antibodies; Anticardiolipin; Pregnancy

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Introduction

Ten percent of all pregnancies are complicated by hypertension. Eclampsia and preeclampsia accounts for half of these cases worldwide [1]. Maternal eclampsia is associated with systemic abnormalities involving the kidneys, liver, or blood and fetal abnormalities as fetal growth restriction, reduced amniotic fluid and abnormal fetal

oxygenation and eclampsia is associated with approximately 13%maternal deaths worldwide [2].

Antiphospholipid antibodies are heterogenous group of autoantibodies directed against anionic phospholipids or phospholipid containing structures [3]. Although these antibodies may be seen in normal pregnancies but there are evidences that these antibodies are associated with complications in pregnancy as preeclampsia, thrombosis, placental infarctions and recurrent fetal deaths. The presence of these antibodies in pregnant women raises the possibility of obstetric and thrombotic complications [4]. The causal relationship between antiphospholipid antibodies and preeclampsia has not yet been proven [5]. The aim of this study is to assess the level of antiphospholipid antibodies in normal pregnancy and in pregnancy complicated by preeclampsia.

Patients and Methods

Study Design & Setting

This study is a case-control study, conducted in the Department of Obstetrics and Gynecology/Baghdad Teaching Hospital/Medical City/during the period from Jan. 2012- January 2013. Seventy-two primigravida pregnant women were enrolled in the study, 48 of them had preeclampsia and 24 had normal pregnancy as a control group.

Inclusion criteria:

The study groups include:

I. Preeclampsia groups; were (48) subdivided into two subgroups:

   First subgroup: included 24 patients diagnosed with severe preeclampsia With blood pressure ≥ 160/110mm Hg and Proteinuria of 2+ or more on two random urine samples collected at Least 4 hours apart.

   Second subgroup: included 24 patients diagnosed with mild preeclampsia with blood pressure ≥ 140/90 mm Hg but less than 160/110 mm Hg and without signs and symptoms that are associated with severe preeclampsia while Proteinuria in this group was 1+ or 2+.

II. *Control groups*: The control group included 24 pregnant women, who have uncomplicated antenatal course. Blood pressure measurements in this group were normal

Exclusion Criteria:

1. Preexisting chronic hypertension
2. Diabetes mellitus
3. Multiple pregnancies
4. Patient with renal disease

Women with infection or other autoimmune disease are not excluded

because the APLAB are cofactor independent in these groups and in our study, we work on APLAB which are cofactor dependent.

Research Plan:

Verbal consents were obtained from all women. Patients and control groups were matched for the age (16-42 years) and gestational age (24-40 weeks).

The gestational age was calculated by weeks of gestation since the last menstrual period and confirmed by early ultrasound. The blood pressure was measured twice 4 hours apart at rest, and the diastolic level was measured at korotkoff phase V.

- ✓ APLAB and ACLAB levels were measured by ELISA using ORGENTEC Diagnostics GmbH (ORG 529 Anti-Phospholipid Screen IgG/IgM) and (Anticardiolipin screen IgG/IgM) kit, according to the manufacturer's instructions.
- ✓ Urine sample were taken for Proteinuria by dipstick.
- ✓ Blood sample for urea, creatinine.

Specimen collection, storage and handling

We collected the whole blood about 5ml, from antecubital vein or from the dorsum of the hand from each patient using acceptable medical techniques to avoid hemolysis. Blood allowed clotting at room temperature for at least 30 minutes and the serum separated by centrifugation for 15 minutes and frozen at -20^{o}C. The serum should appear clear and non-hemolyzed. Enzyme-linked immunosorption assay (ELISA), consisting in outline of adding a sample of patient serum or plasma to plastic micro wheels saturated with bovine beta-2-glycoprotein I. (co factor dependent).

With some particular CL or mixture of PL, then we are measuring how much patient immunoglobulin (Ig) is captured by adding an anti-human IgG, IgM, conjugated with an enzyme that generates a colored product. The intensity of this color is measured photo metrically at 450 nm. The amount of color is directly proportional to the concentration of IgG, IgM antibodies present in the original sample.

Statistical analysis

The significance of difference for more than two groups and using independent student-t-test for difference between two means, while different percentages (qualitative data from different preeclampsia groups and from control group) were tested using Pearson chi-square test (X^2-test). Statistical

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significance was considered whenever the P-value was less than 0.05.

Results

A total of 72 primigravida women in their third trimester of pregnancy were included in our study. The demographic characteristics of the three groups included in the study are not statistically different in the age and gestational age. Table 2 showed distribution of mothers with high titer (more than 50 U/ml) of APLAB among the all groups. In severe preeclampsia group 2 mothers had high titer of APLAB IgG (8.3%), and 5 mothers had high titer of ACLAB IgM (20.8%). And only two mothers in mild group showed high titer APLAB IgG (8.3%), while in control group one mother has high titer APLAB IgM (4.2%). Table 3 shows significant difference between severe and mild preeclampsia group regarding APLAB IgM and between preeclampsia groups and control group regarding APLAB IgG. There is significant difference between the three groups regarding ACLAB IgM and between the severe preeclampsia group and both the mild and control groups regarding ACLAB IgG. Figure 1, showed elevation in ACLAB IgM in preeclampsia group with mean ±SD of (21.93±16.12) IU/L, (8.22±1.75) IU/L for severe and mild preeclampsia respectively while in control group it was (6.62± 2.8). This was highly significant in severe preeclampsia when compared to control group.

Table 1

Gestational age distribution within the groups

GA (weeks)		Severe PE		Mild PE		Control		P value
		No	%	No	%	No	%	
	Preterm	15	62.5	6	25.0	7	29.1	> 0.05
	Full term	9	37.5	18	75.0	17	70.8	
	Mean±SD (Range)	34.5±5.00 (24-40)		36.9±2.48 (29-40)		36.4±3.42 (26-40)		
*Significant using Pearson Chi-square test at 0.05 level								

Preterm birth (between 24+0 and 36+6 weeks). Full term (> 37 weeks)

Table 2

The distribution of APLAB in different groups

		Severe PE		Mild PE		Control	
		No	%	No	%	No	%
APLAB IgM	High	-	-	-	-	1	4.2
	Normal	24	100	24	100	23	95.8
APLAB IgG	High	2	8.3	2	8.3	-	-
	Normal	22	91.7	22	91.7	24	100
AclAB IgM	High	5	20.8	-	-	-	-
	Normal	19	79.1	24	100.0	24	100.0
AClAB IgG	High	-	-	-	-	-	-
	Normal	24	100	24	100.0	24	100.0

Table 3

The mean ±SD of APLAB in three different groups

	Severe PE	Mild PE	Control	Severe X Mild	Severe X Control	Mild X Control
APlAB IgM	11.23±4.48 (6.0-25.0)	8.34±1.98 (4.8-12.0)	8.20±9.17 (3.9-50.0)	0.006*	0.152	0.941
APlAB IgG	13.80±9.97 (4.8-43.1)	11.43±9.74 (4.9-44.2)	6.93±2.84 (3.9-14.3)	0.409	0.002*	0.035*
AClAB IgM	21.93±16.12 (7.8-54.4)	8.22±1.75 (5.1-11.9)	6.62±2.08 (3.8-10.4)	0.0001*	0.0001*	0.006*
AClAB IgG	12.91±7.70 (4.5-30.7)	7.49±1.96 (4.6-11.4)	6.59±2.13 (3.7-10.0)	0.002*	0.0001*	0.134

*Significant using Students-t-test for two independent means at 0.05 level

Data were presented as Mean±SD (Range)EA

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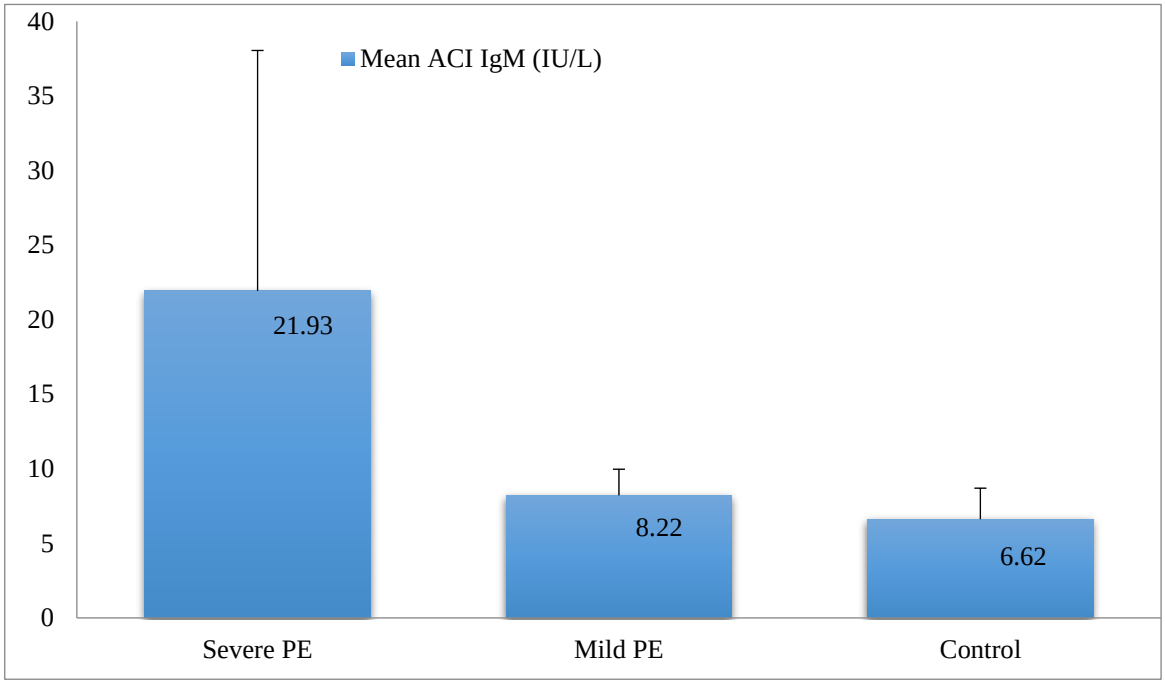

Figure 1

The mean of ACLAB IgM in different groups

Discussion

The relationship between APS and an increased risk of developing preeclampsia is known; however, whether there is an association between high titers of APLAB and preeclampsia in the absence of APS is controversy [6]. In this study, we compared between classes of antiphospholipid antibodies and anticardiolipin antibodies (ACLAB) and we study which one is increased in Preeclampsia. Our study showed significant difference in the level of both classes of ACLAB between the three groups and significant difference in the level of IgG class of APLAB between severe preeclampsia and control group. This finding agrees with other similar studies that demonstrated an increased risk of preeclampsia in the presence of antiphospholipid antibodies [6,7] especially before 34 weeks of gestation as they found that 20% of women with severe preeclamsia < 34 weeks of gestation has high level of APLAB [8]. Other studies failed to show this association and conclude that the prevalence of APLAB among preeclampsia women is low [9,10,11]. Moderate-to-high levels of anticard-iolipin antibodies are associated with

preeclampsia, but there is insufficient evidence to use anticardiolipin antibodies as predictors of preeclampsia in clinical practice [12]. It was believed that these antibodies may activate the coagulation pathway and cause preeclampsia [9,13] and there were environmental and genetic factors predisposes these antibodies to be pathogenic. ACLAB accompany with plasma protein cofactors such as β2-globulin-1, prothrombin, annexin V [12]. C-protein an S- protein are said to have an important role in thrombotic state in eclampsia and preeclampsia [10]. Testing for APLAB in women at risk for preeclampsia was not recommended [5] and some they recommend testing of these antibodies in the presence of history of thrombosis or autoimmune disease [9]. A possible reason for the conflicting data arising from studies of APL AB in preeclamptic women is differing thresholds for a positive test for APLAB and a lack of standardization of the assays used in these investigations. (Controversies in methodological approaches) or the fact that preeclampsia is a multifactorial disorder. Nevertheless, sample size imparts an important role in interpretation of the results. Also, the types of antibodies that used. But till now no agreement regarding what tests which are best suited for screening for APS in both the general and pregnant population

Conclusion

There is significantly elevated level of ACL IgM in severe preeclampsia when compared to normal pregnancy

Recommendations

These findings show the importance of using anticardiolipin antibodies IgM in assessing those with risk factor to develop preeclampsia. Large prospective study is needed to explore their clinical applicability. ACL IgM is available, simple test in assessing of severity of preeclmpsia.

Competing interests

Author declares that they have no competing interests.

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