

Prevalence Of Antiphospholipid Antibodies In Pregnant Women At A Secondary Health Care Institution In Lagos

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Citation

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Abstract

OBJECTIVE: The presence of antiphospholipid antibody has been associated with complications in pregnancy; hence the need to determine the prevalence of this antibody in pregnant women attending the ante-natal clinic in a secondary health care institution in Lagos metropolis using screening tests. **SUBJECTS AND METHODS** A cross-sectional study was carried out on a total of one hundred and thirty one (131) women attending obstetrics and gynaecology clinic at the General hospital Surulere Lagos from 1st to 18th February 2005. Out of these attendees, 37 were non-pregnant, 31 were pregnant with no complications in pregnancy (apparently normal) and 63 pregnant women with complications in pregnancy. Blood samples were collected from ante-natal patients after obtaining informed consent. The plasma of all the samples were subjected to kaolin clotting time and the serum of forty-three (43) of the samples were run on an enzyme-linked immunosorbent assay (ELISA) for the semi quantitative determination of IgG and IgM anticardiolipin antibodies. **RESULTS:** A prevalence of 9.3% was obtained for the antiphospholipid antibodies by the coagulation-based kaolin clotting time, and 2.3% by a more sensitive immunological-based ELISA test on the same set of patients. **CONCLUSION:** In order to avoid false positive results, it is recommended that immunological-based ELISA test be carried out on patients that test positive to kaolin clotting time and/or have clinical features suggestive of the presence of antiphospholipid antibody before they are commenced on therapy.

INTRODUCTION

Antiphospholipid antibodies otherwise known as Lupus anticoagulant because it was first seen in the serum of a woman with systemic Lupus erythematosus in 1952, is now known to be present in a variety of other clinical conditions such as recurrent venous and arterial thromboembolic disorders, habitual abortion, abruptio placenta, pre-eclampsia and auto immune disorders.

All cell surfaces have phospholipid on the endothelium which can be damaged by Anti-phospholipid antibody. When endothelial cells are damaged, they do not function normally and thrombotic complications can occur. The antibodies are usually IgG, IgM, or a mixture and rarely IgA antibodies may be found.

There are various screening test available for antiphospholipid antibodies such as Activated partial prothrombin time (APPT), thromboplastin inhibition test [TTI], kaolin clotting time [KCT], and Russel's viper venom time, [dRVVT] among others. However, no consensus has

been reached as to the most reliable test.

There is an association between antiphospholipid antibodies and pregnancy loss and diverse disease conditions. The prevalence of Lupus antibody (LA) among Nigerians with recurrent foetal loss has been estimated to be 4.3%.¹ A Study conducted in Benin showed a prevalence of 15.4% in pre-eclampsia, 2% among apparently healthy pregnant women and 8% among non-pregnant multiparous women.² It is worthy of note that these studies were conducted using coagulation screening techniques to determine presence or absence of Lupus Anticoagulant. This study utilized a combination of coagulation screening tests and the standard immunological test for IgG and IgM (aCL) antibody as recommended by international guideline³ determining the prevalence of these antibodies in normal and problematic pregnancies.

ANTICARDIOLIPIN IGG/IGM SEMIQUANTITATIVE TEST KIT

This is an enzyme-linked immunosorbent assay (ELISA) for

the semiquantitative determination of IgG and IgM anticardiolipin antibodies in human serum or plasma. It is meant to detect and semi-quantify anticardiolipin antibodies in those with SLE and lupus-like disorders (antiphospholipid syndrome).

KAOLIN CLOTTING TIME

When an assay on a range of mixture of normal and patient's plasma is done, the presence of Lupus anticoagulant deficiency is indicated by the different patterns of response obtained. ⁴

METHODS

A cross-sectional study was performed at the obstetrics and gynecology clinic of General Hospital Surulere, Lagos, which is a secondary health care institution that registers an average of 50 patients per day. The study was conducted between 1st to 18th February 2005 after obtaining the institution's research and ethics committee's approval

SUBJECTS

Three groups of women were recruited

1. Women in reproductive age groups who were not pregnant.
2. Apparently normal pregnant women.
3. Pregnant women with complicated pregnancies such as:
 - a. Recurrent fetal loss; defined as three or more spontaneous abortions at less than ten weeks gestations. Ultrasound confirmed gestation or pregnancy test by HCG.
 - b. History of pre-eclampsia.
 - c. History of fetal or premature delivery before 34 weeks of gestation.
 - d. Neonatal death after delivery at less than 34 weeks of gestation

Each patient's data and blood samples were assigned the same code number and the following information gathered after an informed consent was sought and obtained from the patient.

1. Age.
2. Gravidity.
3. Last menstrual period or Gestational age as appropriate.
4. Drug history apart from the usual ANC drugs
5. Medical history.

COLLECTION OF SAMPLES

A total of 10mls of venous blood was collected from each

subject. of which 3.5mls was put in EDTA specimen bottle, 4.5 mls put in a citrated bottle and 2ml in a plain bottle. The citrated bottle was used for the coagulation studies on the same day of collection viz; KCT. The EDTA sample was used for full blood count on collection day using the Advia 60, while the plain bottle sample was used for immunological studies. Samples for immunological assay were separated on the day of collection and stored at below minus 20 degrees until all were ready for ELISA.

KAOLIN CLOTTING TIME

Normal and patient's plasma are mixed in plastic tubes in the following ratios of normal to patient's plasma:

10:0,9:1,8:2,5:5,2:8,1:9,and 0:10. 0.2ml of each mixture is pipetted into a glass tube at 37 ° C. 0.1ml of Kaolin is added and incubated for 3 min, then 0.2ml of CaCl₂ added the clotting time is recorded.

RESULTS

For a positive result, the ratio shown below should be 1.2 or greater ⁴.

KCT (80%N: 20%Test) \geq 1.2

KCT (100% N)

A control KCT of <60s may indicate contamination of the control plasma with phospholipids.

KCT---Kaolin Clotting Time.

N---Normal control sample.

Test-Test sample

RESULTS

A total of hundred and thirty one (131) patients were bled for this study. Consisting of three groups of patient, namely, sixty-three (63) patients with complications in pregnancy, thirty one (31) patients with normal pregnancy and thirty seven (37) non- pregnant controls. All subjects were females.

About 46 of the 63 patients (73%) with complications in pregnancy had pregnancy induced hypertension, 8 (12.69%) had recurrent abortion and 3 (4.76%) had intrauterine death as complication. Most (10) of the 11 patients (90.9%) had pregnancy induced hypertension and 1 (9%) had recurrent abortion. Using Kaolin Clotting time ratio,(Table 1) 11 of the 63 patients (17.46%) with complications in pregnancy were positive to kaolin clotting time ratio.Two of the 31 (6.45%) with normal pregnancy and 3 of the 37 (8.11%) who were non-pregnant also tested positive to the kaolin clotting time.

Using a more specific immunological assay of IgG, a total of 43 patients were studied (Table 2) of which 4 (9.3%) showed positivity to Kaolin clotting time as compared with 1 (2.3%) when IgG antiphospholipid assay was done on the same set of 43 patients.

In IgM assay all cases were negative.

None of the 131 patients bled admitted to the use of drugs that could affect the result. Only 3 of the 131 admitted to history of psychiatric illness. Approximately 3% of the 131 patients reported recent infection and they were all non-pregnant patients. None of the 131 reported history of autoimmune disease.

Figure 1

Table 1: Groups of respondents and their kaolin clotting time ratio

The three groups of patients * Kaolin clotting time ratio Crosstabulation

Count		Kaolin clotting time ratio		Total
		negative	positive	
The three groups of patients	complicated pregnancy	52	11	63
	Normal pregnancy	29	2	31
	Non-pregnant females	34	3	37
Total		115	16	131

Figure 2

Table 2: Kaolin clotting time ratio and OD_IGGAPA_

Crosstab					
			OD_IGGAPA		Total
			negative	positive	
Kaolin clotting time ratio	negative	Count	38		38
		% of Total	88.4%		88.4%
	positive	Count	4	1	5
		% of Total	9.3%	2.3%	11.6%
Total		Count	42	1	43
		% of Total	97.7%	2.3%	100.0%

OD IGG APA: Optical density IgG Antiphospholipid antibody

DISCUSSION

The finding of positive kaolin clotting time ratio in 2 and 3 of normal pregnancy and non-pregnant females respectively is in keeping with works of Creagh et.al.who detected lupus anticoagulant or anticardiolipin in 3% of 500 normal pregnant women. ⁵ Such incidental autoantibodies may not be associated with increased risk of arterial thrombosis or miscarriage, ⁶⁷ even though high titre IgG anticardiolipin has been linked to future venous thrombosis in an epidemiological study. A percentage of 9.3 was obtained for

patients with complications in pregnancy in this study using the screening test i.e. kaolin clotting time. This is comparable with 4.3% obtained for patients with recurrent fetal loss, and 15.4% in pre-eclamptic patients in the Benin study. ¹²

However, using a more sensitive immunological assay of anticardiolipin antibody assay (IgG) only 2.3% of the patients bled tested positive. The only patient who had antiphospholipid antibody had pregnancy induced hypertension. Hence the combined use of coagulation-based i.e kaolin clotting time, and immunological-based assay i.e anticardiolipin assay is essential.

Various drugs associated with clinical features of antiphospholipid antibody e.g. Chlorpromazine, hydralazine, quinidine, phenytoin, and various antibiotics did not affect this result because all the patients reported not to be on this drugs. Psychiatric illness e.g. multi infarct dementia, migraine also associated with antiphospholipid antibody did not significantly affect the result because only one of the 131 bled admitted to history of psychiatry illness. Autoimmune disorders did not also affect the result because non of the patient had autoimmune disorders. Secondary antiphospholipid syndrome has been associated with rheumatic and connective tissue disorders where thrombosis, recurrent miscarriage, or both occur in association with antiphospholipid antibodies in systemic lupus erythromatosus, rheumatoid arthritis, systemic sclerosis, psoriatic arthropathy and Bechcet syndrome.

Only 3% of the 131 patients in the study reported recent infections, and all were non-pregnant females, this has not affected the result significantly.

CONCLUSION

It is obvious from this study that many patients are wrongly diagnosed as having antiphospholipid antibody, particularly if only the screening test is used to make the diagnosis i.e Kaolin clotting time, activated partial prothrombin time, prothrombin time, dilute Russel Viper Venom test. There is need to include more sensitive screening tests such as immunological-based assay in confirming the presence of either IgG or IgM antiphospholipid antibody before patients are commenced on heparin. This is important to avoid unnecessary complications associated with the use of heparin.

However, low dose aspirin may be commenced on patients

that test positive to the screening test and/or have clinical features suggestive of antiphospholipid antibody.

Further work is still needed to elucidate the importance of immunological-based assay over the coagulation-based assay, and to confirm the actual prevalence of antiphospholipid antibody.

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