MEASUREMENT OF ANTICARDIOLIPIN ANTIBODY BY ELISA WITH ANTICARDIOLIPIN ANTIBODY COFACTOR IN PREECLAMPSIA


1) Department of Obstetrics and Gynecology, Teikyo University School of Medicine, Tokyo 173, Japan
2) Yamasa shoyu Co., Ltd., Choshi 288, Japan

INTRODUCTION

The presence of anticardiolipin antibody (ACA), anti-DNA and anti-laminin antibody was reported in preeclampsia. Recently McNeil and Matsuura reported that highly purified ACA does not bind to the cardiolipin (CL) antigen when it is assayed by a modified CL ELISA in which the blocking agent does not contain bovine serum. Binding to the CL antigen will only occur if normal human plasma, human serum or bovine serum is present, suggesting that the binding of ACA to CL requires the presence of a plasma/serum cofactor. Matsuura has purified this cofactor and shown that the binding of ACA to CL requires the presence of this cofactor in dose-dependent manner. Moreover the ACA binds to CL/cofactor complex antigen. He also developed the new ELISA in which contains cofactor. Using this ELISA, we tried to find the presence of ACA which bind to the CL/cofactor complex in preeclampsia, and its clinical significance, whether maternal and fetal complications are present or not in ACA positive cases in preeclampsia.

MATERIALS AND METHODS

Serum samples were taken from 38 cases of pure type of preeclampsia including 23 cases of severe type from 24 to 40 weeks gestation, and 21 normal pregnant women from 20 to 40 week of gestation.

ACA was measured with ELISA which was developed by Yamasa Shyou Co. Ltd. (Fig 1). Cardiolipin was added to each well and coated onto the well surface in microplate by evaporation. Coated wells were blocked with 1% BSA-PBS for 1 hour. After washing the wells
with 200µl of Tween BSA-PBS. 50µl of cofactor solution was added into wells and incubated for 10 min. at room temperature. 50µl of standards or diluted samples of 1:100 in BSA-PBS were added and incubated for 30 min. at room temperature.

After washing, 100µl of HRP conjugated anti human IgG was added into wells and incubated for 30 min. at room temperature. For colour developing, 100µl of tetramethylbenzidine was added. Optical density was measured at 450nm. Five serum ranging from 1.3 U/ml to 125 U/ml were used as a standard of this measurement.

RESULTS

1. Measurements by the ELISA without cofactor
None of 21 normal pregnant women showed positive ACA. The levels of ACA in normal pregnant women were 2.34U/ml in average and the standard deviation was 1.46U/ml. More than 6.7 U/ml which is value above or equal to mean plus 3 standard deviation was considered positive. In mild cases of preeclampsia, ACA was positive in 2 out of 15 cases(13.3%). In severe cases ACA was positive in 2 out of 23 cases(8.7%). The positive percentage of All of preeclampsia was 10.5%.

2. Measurements by the ELISA with cofactor.
The levels of ACA in normal pregnant women were 0.54U/ml in average and the standard deviation was 0.58U/ml. More than 2.3 U/ml which is value above or equal to mean plus 3 standard deviation was considered positive.

In mild cases of preeclampsia, ACA was positive in 3 out of 23 cases(20%). In severe cases ACA was positive in 5 out of 23 (21.7%). The positive percentage of overall preeclampsia was 21%(Fig 2).

The positive percentage of ACA by ELISA with cofactor was greater than that by ELISA without cofactor.

3. Clinical finding of ACA positive cases by ELISA with cofactor
Seven of 8 cases had proteinuria. Intrauterine growth retardation(IUGR) was found in 4 of 9 fetuses. Two of the 8 cases showed fetal distress. One patient developed eclampsia. Three of them showed low platelet count. In 2 of the 6 cases, prolonged activated partial prothrombin time (APTT) was found. In preeclampsia, relationship between ACA positive cases and IUGR was calculated. The incidence of IUGR was higher than that of the ACA negative case. In only mild
15 cases, the value was statistically significant.

DISCUSSION

Antiphospholipid antibody cofactor is a glycoprotein including carbohydrate (content 19%). It was known as the β2-glycoprotein I or apolipoprotein H. It is one of β2-globulin with molecular weight of 50 kilodalton. It is composed of 326 amino acid and has a unique sequence with abundant proline residues, and multiple disulfide bridges. The concentration of ACA cofactor in plasma is -200μ g/ml and 40% is associated with lipoproteins of various classes. It is reported to bind to lipoprotein, anionic phospholipid, platelets, heparin, DNA and mitochondria. Physiologic function of ACA cofactor has been found to inhibit the intrinsic coagulation pathway and to bind to platelets and inhibit of ADP-mediated platelet aggregation. It was suggested that ACA cofactor may have the effect to diminish unwanted activation of blood coagulation by binding to and neutralizing negatively charged macromolecules. Anticardiolipin antibodies interfere with ACA cofactor function.

Several reports have suggested an association between antiphospholipid antibodies and preeclampsia. Branch suggests that a significant proportion of women with early onset of severe preeclampsia has antiphospholipid antibodies. Incidence of ACA by ELISA with cofactor in our cases was 10.5 % in mild type, 21.7% in severe type and 20.0% in total cases.

Harris confirmed a close correlation between ACA and lupus anticoagulant. Prolonged APTT was found in 2 of 6 cases. The presence of lupus anticoagulant in those cases was considered. IUGR were found more frequently in ACA positive cases, especially in a mild type. Vascular constriction and thrombosis are related with the cause of IUGR. Prostacyclin is a potent vasodilator and an inhibitor of platelet aggregation. Inhibition of prostacyclin production by ACA is suggested. By pathological examination of the placenta delivered from two ACA positive patients, infarction, calcification and fibrinoid necrosis were detected. Wolf also reported the presence of massive infarction, fibrinoid necrosis and intraluminal thrombosis of the placenta. These findings may play a role in the pathogenesis of IUGR.

We suggest the ACA which binds to cardiolipin/cofactor complex also relates to the clinical abnormality such as IUGR and thrombosis. We recommend that
severe preelampsia be screened for ACA. If ACA is identified, it is appropriate to consider anticoagulant therapy such as aspirin or heparin.

REFERENCES

**Fig 1**
Measurement of Anti-cardiolipin Antibody using ELISA with Cofactor

- **Microplate Coating:** Cardiolipin was added to each well and coated onto the well surface by evaporation. Coated wells were blocked with 1% BSA-PBS for 1 hour.

- **Formation of Complex:**
  - Wash the wells three times with 200 μl of Tween BSA-PBS
  - Dispense 30 μl of cofactor solution
  - Incubate for 10 min. at room temperature

- **First Reaction:**
  - Dispense 30 μl of standards or diluted samples of 1:100 in BSA-PBS
  - Incubate for 30 min. at room temperature
  - Wash the wells three times with Tween BSA-PBS

- **Second Reaction:**
  - Dispense 100 μl of HRP conjugated anti-human IgG
  - Incubate for 30 min. at room temperature
  - Wash the wells three times with Tween BSA-PBS

- **Color Developing Reaction:**
  - Dispense 100 μl of tetramethylbenzidine
  - Incubate for 10 min. at room temperature
  - Dispense 100 μl of H2SO4

Optical density was measured at 450 nm.

**Fig 2**
Levels of anti-carodiolipin antibody using ELISA with cofactor

[Graph showing levels of anti-carodiolipin antibody]