妊娠中毒症における抗カルジオリピン抗体の存在

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Anticardiolipin antibody in preeclampsia

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[Summary]

The presence of anticardiolipin antibody (ACA) in preeclampsia and eclampsia, and its clinical significance were studied. Serum samples were taken from 57 cases of preeclampsia including 29 cases of the severe type and 37 normal pregnant women ranging from 28 to 40 weeks of pregnancy. ACA was measured by an enzyme-linked immunosorbent assay using Harris' standard. Values above or equal to the mean plus 3 standard deviations were considered positive. The mean values and standard deviation of 41 normal pregnant women was 4.3 and 3.2 U/ml respectively. In all cases of preeclampsia, ACA was positive in 7 of 57 cases (12.3%). In mild cases of preeclampsia, ACA was positive in 3 of 28 cases (10.7%). In severe cases ACA was positive in 4 of 29 (13.8%). Three cases of 7 ACA positive fetuses delivered light for date neonates. Four fetuses suffered from fetal distress. Activated partial thromboplastin time (APTT) was measured in 5 cases. In 2 of them (40%), APTT was prolonged. Thrombocytopenia (less than $15 \times 10^4/\text{cmm}$) was found in 3 of 7 cases (43%). Incidence of intrauterine growth retardation (IUGR) in preeclampsia was 21.1%. Higher incidence of IUGR was found in ACA positive (42.9%) than in ACA negative preeclampsia (18.0%).

We suggest that ACA appears in some cases of preeclampsia, and that ACA may be associated with clinical abnormalities such as IUGR and coagulation disturbances in preeclampsia.

Key words : anticardiolipin antibody,
preeclampsia,
autoantibody

【概 要】

妊娠中毒症における抗カルジオリピン抗体 (ACA) の存在とその意義につき検討した。妊娠中毒症 57 例 (重症型 29 例, 軽症型 28 例) と 28 週から 40 週までの正常妊娠 37 例を対象とし, ACA の測定は ELISA 法を用いた。標準血清は, Harris らの標準品の希釈試験データより設定された血清を使用した。正常妊娠の平均 $+3 \times$ 標準偏差以上を陽性とした。正常妊婦の平均値は 4.3 U/ml , 標準偏差は 3.2 U/ml であった。妊娠中毒症 57 例中 7 例 (12.3%), 軽症型では 28 例中 3 例 (10.7%), 重症型 29 例では 4 例 (13.8%) が陽性であった。ACA の高値を示し分娩に至った 7 胎児のうち 3 例が低出生体重児であった。activated partial thromboplastin time (APTT) を測定し得た 5 例中 2 例に延長を認めた。血小板減少は 7 例中 3 例に認められた。子宮内胎児発育遅延は ACA 陰性群の 18.0% に比し, ACA 陽性群では 42.9% と ACA 陽性群で高率であった。妊娠中毒症においても抗カルジオリピン抗体が出現することがあり, 陽性例では子宮内胎児発育遅延や凝固異常を伴うため, 妊娠中毒症においても抗カルジオリピン抗体の検討をする必要があると思われた。

I. INTRODUCTION

The presence of anticardiolipin antibody (ACA) has been reported in collagen disease, habitual abortion of unknown etiology and unexplained intrauterine death^{1,2)}. As a possible pathophysiology in preeclampsia, immunological alteration has been hypothesized. Recently in preeclampsia various kinds of autoantibodies, such as antibodies for smooth muscle and vascular endothelial cells were detected^{3,4)}. ACA, which is an antiphospholipid antibody, is also an autoantibody. Inhibition of prostacyclin production by ACA in endothelial cells is suggested⁵⁾. The disturbance of prostacyclin production by endothelial cell, obtained from preeclamptic patients, also has been reported⁶⁾. We measured ACA in preeclampsia and eclampsia, and to determine its clinical significance we studied whether or not maternal and fetal complications were present in ACA positive cases.

II. MATERIALS AND METHODS

Serum samples were taken from 57 cases of preeclampsia including 29 cases of the severe type and 37 normal pregnant women ranging from 28 to 40 weeks of gestation. Only one sample was measured by duplicate in each case. The maternal ages of both groups were not statistically different. The classification of preeclampsia by Japanese Society of Obstetrics and Gynecology was used. The criteria

for severe preeclampsia was a blood pressure of 160 mmHg systolic and 110 mmHg diastolic on two occasions at least 6 hours apart; proteinuria of at least 2 gm/24 hours or general edema.

Clinical findings of ACA positive cases (Table 1)

Five of 7 positive women were primigravida. One multipara patient had some problems associated with previous pregnancies such as preeclampsia with IUGR. Another multiparous woman had spontaneous abortion in her first pregnancy. Preterm delivery was found in 3 cases. Three of 7 fetuses delivered as light for date neonates. Four experienced fetal distress. One patient developed eclampsia. Another displayed the HELLP syndrome.

ACA measurement

ACA was measured with an enzyme-linked immunosorbent assay (ELISA) which was developed by the Medical Biological Laboratory (MBL) (Nagoya, Japan). Cardiolipin coated microcups were set into the microplate holder. Serum or standard ($100 \mu\text{l}$) diluted 1:101 in sample buffer (in duplicate) were added to test wells. The microcups were incubated for 1 hour at room temperature and washed four times with phosphate buffered saline (PBS). The peroxidase conjugated anti-human IgG/F (ab')₂ rabbit antibody (MBL) was added to each well and the microcups were incubated for 1 hour. The microcups were washed four times with

Table 1 Clinical findings of the cases with positive anticardiolipin antibody (ACA) in preeclampsia

ID	ACA U/ml	Age	Gravidity Parity	Types	Delivery week	Baby weight	Apgar score	Others
MK	17	37	0 G 0 P	pH	32	1258*	4	FD, HELLP syn.
SM	40.5	32	2 G 1 P	ePH	31	1248*	2	FD, eclampsia
NT	15.8	35	1 G 1 P	ePH	34	1652*	8	FD
TM	19.5	24	0 G 0 P	H	38	2802	10	premature labor
HM	19	32	0 G 0 P	ph	39	3090	10	FD
HY	24	25	0 G 0 P	ep	39	2788	9	
KM	16	30	0 G 0 P	eph	39	2610	9	

ACA=anticardiolipin antibody, FD=fetal distress,

* Intrauterine growth retardation.

Fig 1 Standard Curve for Anti-Cardiolipin Antibody Measurement

PBS. One hundred microliter of o-phenylenediamine was added to each well and incubated for 15 minutes. The reaction was stopped by addition of 2 N H_2SO_4 to wells. The optical absorbance of each well was read at 492 nm with MPRA 4 (TOYO SODA). Five sera ranging from 7.5 U/ml to 120 U/ml adjusted with the Harris' standard were used as standards for this assay⁷⁾. The data shown in Fig 1 demonstrate that optical density is proportional to the concentration of anticardiolipin antibody. The assay results were expressed in standard units. Values above or equal

to the mean plus 3 standard deviation (SD) were considered abnormal or positive.

Anti ssDNA antibody measurement

Anti ssDNA antibody was measured using an ELISA kit (MBL). Positive values over than 2 U/ml which is the values above mean plus 2 SD of healthy non pregnant women was considered positive.

Assay of complement components

CH 50 was measured by the Mayer method⁸⁾. Complement component C 3, C 4 were quantified by rocket electrophoresis by means of standard routine techniques⁹⁾.

III. RESULTS

1. The level of ACA in normal pregnancy and preeclampsia

The mean level and standard deviation of mean of 37 normal pregnant women was 4.3 U/ml and 3.2 U/ml respectively (Fig 2).

In our series of preeclampsia, ACA was positive (higher than 14 U/ml) in 7 out of 57 cases (12.3%) (Fig 2). ACA was positive in 3 out of 28 mild cases (10.7%) and in 4 out of 29 severe cases (13.8%). No positive case was found in normal pregnant women.

2. Laboratory data in ACA positive cases

(Table 2)

The antissDNA antibody was measured in 5 cases. Among them 3 (60%) were positive. Incidence of antissDNA antibody in 15 ACA negative cases was 20% (3/15). Incidence of antissDNA antibody in ACA positive cases was significantly higher than that of negative cases ($P < 0.05$).

Fig 2 Levels of anti-cardiolipin antibody in normal pregnancy and preeclampsia. The dotted line indicates the upper limit of normal value (mean + 3 SD)

Activated partial thromboplastin time (APTT) was measured in 5 cases. In 2 out of these cases (40%), APTT was prolonged. In severe preeclampsia the incidence of prolonged APTT in ACA positive cases is higher than that of 10 negative cases (20%) ($p < 0.05$).

Thrombocytopenia (less than $15 \times 10^4/\text{cmm}$) was found in 3 of 7 cases (43%). Incidence of thrombocytopenia in ACA positive cases was higher than that of 15 negative cases (20%). No remarkable change was found in 4 cases which were determined for complement (CH 50, C 3, C 4). Umbilical arterial pulsatility index value is calculated from the flow velocity wave by pulsed doppler ultrasound device¹⁰. High values of pulsatility index were detected in 1 out of 4 case.

3. Relationship between ACA and Intrauterine growth retardation (IUGR) (Table 3)

The evaluation of IUGR was done by Nishida's definition¹¹. Incidence of IUGR in all cases of preeclampsia was 21.1%. Its incidences in ACA positive and ACA negative cases were 42.9% and 18.0% respectively. Especially in severe cases, its incidences was 75% in ACA positive cases and only 28.0% in ACA negative. Incidence of IUGR was higher detected in ACA positive cases than ACA negative cases in overall and severe cases ($P < 0.05$ in both cases).

Table 2 Laboratory data of the cases with positive anticardiolipin antibody in preeclampsia

	ACA U/ml	ssDNA U/ml	APTT Sec.	Platelet $10^4/\text{cmm}$	CH 50	C 3	C 4	UmPI
MK	17		<u>37.1</u>	<u>2.2</u>	—	—	—	—
SM	40.5	—	<u>36.8</u>	<u>10.5</u>	—	—	—	—
NT	15.8	<u>4.8</u>	34.8	24.5	31	64	29	<u>1.7</u>
TM	19.5	<u>2.6</u>	—	33.6	—	—	—	0.79
HM	19	1.0	—	21.3	—	—	—	—
HY	24	<u>2.5</u>	32.4	35.1	39	85	22	0.8
KM	16	0.9	34.1	<u>9.4</u>	41	77	23	0.75

ACA=anticardiolipin antibody, APTT=activated partial thromboplastin time, UmPI=Umbilical arterial pulsatility index, The abnormal values were underlined.

Table 3 Relationship between positive anticardiolipin antibody (ACA) and intrauterine growth retardation (IUGR) in preeclampsia

	total 57 cases		severe 29 cases		mild 28 cases	
	IUGR+	IUGR-	IUGR+	IUGR-	IUGR+	IUGR-
ACA positive	3	4	3	1	0	3
ACA negative	9	41	7	18	2	23

total cases and severe cases $P < 0.05$, mild cases $P > 0.05$ (χ^2 analysis)

Fig 3 Relationship between the levels of anticardiolipin antibody (ACA) and antissDNA antibody. The dotted lines indicate the upper limit of normal values

4. Relationship between the values of ACA and antissDNA antibody (Fig 3)

Both values of ACA and antissDNA antibody were measured using the same samples in 22 preeclampsia patients. No correlation was detected ($r = 0.04$).

IV. DISCUSSION

The assay for antiphospholipid antibody has been done by various procedures such as radioimmunoassay and ELISA^{1,2)}. In this experiment, the ELISA procedure developed by Medical Biological Laboratory (MBL) was used. This procedure is a modification of ELISA described by Lockshin et al³⁾. By using Harris' standard, it is possible to standardize the anticardiolipin test and for some laboratories to compare their results. In previous experiments the value of 50 healthy nonpregnant women were 6.56 U/ml in mean and 2.54 U/ml in

standard deviation. The values above the mean value plus 3 SD were arbitrarily considered to be abnormal. The positive level of ACA was defined as more than 15 U/ml in non pregnancy. However in pregnant women, the positive level was defined as more than 14 U/ml because the mean value plus 3 SD of ACA values in pregnant women was 13.9 U/ml. Several reports have suggested an association between antiphospholipid antibodies and severe preeclampsia. Branch et al have suggested that a significant proportion of women with early onset of severe preeclampsia have antiphospholipid antibodies¹²⁾. However, Scott reported that ACA was not associated with preeclampsia¹³⁾. Incidence of ACA in our study was 10.7% in the mild type, 13.8% in the severe type and 12.3% in total. Incidence of early onset preeclampsia was 16.7%. This finding was similar to the results reported by Branch et al¹²⁾.

The presence of autoantibodies such as ACA and antiDNA antibody may relate to pathogenesis of preeclampsia. Autoantibody production may be caused by immunological alteration, such as polyclonal B cell activation. The binding of ACA to vascular endothelial cells and platelets may cause vascular constriction and platelet agglutination.¹⁴⁾ Prostacyclin is a potent vasodilator and an inhibitor of platelet aggregation. Inhibition of prostacyclin production in vascular endothelial cells by ACA is suggested to be the mechanism of vascular constriction and platelet agglutination.

A question arises whether ACA is transient or not. One case, which showed positive ACA (15.8 U/ml) transiently during pregnancy, returned to normal levels of 0.75 and 1.0 U/ml at 2 and 4 weeks after delivery, respectively.

However sequential analysis of ACA in preeclampsia is needed to further elucidate this problem.

IUGR were found more frequently in ACA positive cases, especially in the severe type. Vascular constriction and thrombosis are related with the incidence of IUGR. By histological examination of the placentas obtained from three 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 in ACA positive case¹⁵⁾. These changes may cause the increased placental vascular resistance and disturb the placental circulation. As a result, placental functions are depressed and fetal growth is suppressed. These findings may play a role in pathogenesis of IUGR. These courses may be expressed as the increased value of pulsatility index (PI) in the umbilical artery. The increased PI value means the increased vascular resistance of placental vasculature¹⁶⁾.

Lockshin reported ACA positive cases during pregnancy were associated with fetal loss and fetal distress²⁾. In our series, 4 of 7 ACA positive pregnancies developed fetal distress. Placental pathology was evaluated in two of them. Fibrinoid necrosis and villous ischemic changes were detected in the placenta. The high incidence of fetal distress may be due to depressed placental function caused by placental arterial thromboses and villous ischemic change.

It was reported that women with severe preeclampsia and antiphospholipid antibody may experience life threatening thromboembolisms¹²⁾. We did not experience any thromboembolisms in our series.

Prolonged APPT was found in 2 of 5 cases. Lupus anticoagulant (LAC) was detected in those 2 cases. There is a strong correlation between the presence of the LAC and elevated titers of ACA¹⁾. The frequent association of the lupus anticoagulant and elevated titers of ACA strongly suggests that patients with the lupus anticoagulant produce a family of antiphospholipid antibodies. More than 90% of patients with LAC have ACA¹⁷⁾. The pres-

ence of LAC in preeclampsia has been reported by some authors. Lubbe et al reported on a case which was complicated hepatic and splenic haemorrhage¹⁸⁾. Kochenour et al reported on a case which was complicated with pleuropulmonary disease, fever and cardiac manifestation during the puerperium¹⁹⁾.

Decreased platelet counts in ACA positive cases may also rely on the presence of ACA and LAC. These findings have already been reported in cases of systemic lupus erythematosus by Harris and Lockshin^{1,2)}. Harris reported that 11 of 13 (85%) cases, which had platelet counts below $10^5/\text{cmm}$, had raised ACA levels and that there was a significant relation between thrombocytopenia and IgG ACA levels. Platelet adhesion and activation by ACA is thought to be the mechanism of decreased platelet counts¹²⁾.

Several reports have suggested cross-reactivity between antiDNA antibody and ACA²⁰⁾. We detected a high incidence of antissDNA antibody in preeclampsia²¹⁾. AntissDNA antibody was measured in 22 specimens of serum in which ACA had previously been determined. However we could not confirm any correlation between ACA and antiss DNA antibody. We think that the detection of ACA is not based on cross-reactivity with anti DNA antibody.

We suggest that ACA appears in some case of preeclampsia, and that ACA relates to clinical abnormalities such as IUGR and coagulation disturbances in preeclampsia. Treatment with prednisolon, minutes doses of acetylsalicylic acid and human immunoglobulin in ACA or lupus anticoagulant positive cases has resulted in living neonates^{18,22)}. We recommend that preeclampsia should be screened for ACA. If ACA is positive, it is recommended to consider anticoagulant therapy such as aspirin or heparin.

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