Anti-phosphatidylserine-prothrombin antibody in patients with recurrent abortion and preeclampsia

Mari Mitsui, Miwako Yamashiro and Tatsuo Yamamoto

Department of Obstetrics and Gynecology, Nihon University School of Medicine

(Received September 22, 2004)

summary

Aim: The aim of the study was to describe the frequency of anti-phosphatidylserine-prothrombin antibody (aPS/PTAb) in patients with recurrent abortion and preeclampsia, and to study the relationship between the presence of aPS/PTAb and clinical finding. Eighty six cases of recurrent abortion and 82 cases of preeclampsia were studied. A aPS/PTAb was measured by an enzyme linked immunosorbent assay (ELISA).

Results: In patients with recurrent abortion, 3 out of 86 cases (3.4%) were positive in IgG antibody measurements and 5 out of 86 (5.8%) were positive in IgM antibody measurements. In patients with preeclampsia, 2 out of 82 cases (2.5%) were positive in IgG antibody measurements and 13 out of 82 (16%) were positive in IgM antibody measurements. The positive rates of aPS/PTAb in severe hypertension-positive cases is greater than in hypertension-negative cases (p=0.045). The positive rates of aPS/PTAb is higher tendency with in severe type than in mild type (p=0.117). The positive rates of aPS/PTAb is higher tendency with proteinuria and/or hypertension than without proteinuria (p=0.098) or hypertension (p=0.096).

Conclusion: We found that aPS/PTAb appears in some cases of patients with recurrent abortion and preeclampsia. Our data suggest that aPS/PTAb might be a risk factor in patients with recurrent abortion, and may relate to clinical finding in preeclampsia.

Key words—anti-phospholipid syndrome; anti-phosphatidylserine-prothrombin antibody; recurrent abortion; preeclampsia

Introduction

Anti-phospholipid antibodies are a family of antibodies that include the lupus anticoagulant (LA) and anti-cardiolipin antibodies defined by Sapporo criteria. Prothrombin was first described as a cofactor for a circulating anticoagulant in patients with systemic lupus erythematosus (SLE) by Loeliger et al. Prothrombin binds to negatively charged phospholipids through γ-carboxyglutamic acids (GLA) domains. The LA activity was also identified as a binding reactivity to phospholipid-protein complexes, especially complexes of phospholipids and prothrombin. There are 2 kinds of methods to detect anti-prothrombin antibodies.

Recently, it has been reported the enzyme-linked immunosorbent assays (ELISA) that detect of antibodies (aPS/PTAb) against phosphatidylserine (PS)–prothrombin (PT) complex correlate more significantly with the clinical manifestations of antiphospholipid antibody syndrome (APS) in systemic autoimmune disease patients, than do the ELISA detecting antibodies directed to prothrombin bound on irradiated plates.

The association of aPS/PTAb with venous or arterial thrombosis has been reported. Galli et al. have reported the literature regarding aPS/PTAb as a diagnostic criteria for arterial and venous thrombosis in the APS. The presence of APAs has been reported in habitual abortion of unknown etiology and unexplained intrauterine fetal death.

By histological examination of the placentas obtained from preeclampsia patients, infarction and fibrinoid necrosis have been detected. These changes seem to be similar to the findings concerning placenta taken from APA-positive pregnancies. APAs have been known to bind to vascular endothelial cells and platelets. It has been proposed that the functions of APAs are to prevent protein C activation and to enhance platelet aggregation which might cause abnormal coagulation and platelet agglutination without changes in prostacyclin production from endothelial cell.

If aPS/PTAb have the ability to bind villous trophoblasts, aPS/PTAb might cause placental dysfunctions.

We tried to find the frequency of aPS/PTAb in...
patients with recurrent abortion and preeclampsia and to study the relationship between the presence of aPS/PTAb and clinical findings.

**Materials and Methods**

1. **Sample collection**

   Serum samples were taken from 86 cases of recurrent abortion, including 52 cases of the experience of 2 miscarriages, 34 cases of 3 times and more recurrent miscarriages, and from 47 healthy nonpregnant women. In study cases, uterine abnormalities, infection, abnormal antibodies involving blood types and chromosomal abnormality were excluded. Eight cases of systemic autoimmune disease patients were included. Eighteen cases showed APA positive such as anticardiolipin antibody, anti-β2-GPI antibody and LA.

   In preeclampsia study, serum samples were taken from 82 cases of preeclampsia, including 51 cases of the severe type, 31 cases of the mild type and from 40 normal pregnant women of 20 to 40 weeks of gestation. The grade of preeclampsia was classified according to the criteria of the Japanese Society of Obstetrics and Gynecology. No significant difference between 82 cases of preeclampsia and 40 cases of normal pregnancies was found in age, gravity and gestational week.

2. **Measurement of anti-phosphatidylserine-prothrombin antibody (aPS/PTAb)**

   A aPS/PTAb were measured by ELISA developed by Matsuda et al.\(^{13}\) PS (L-α-phosphatidy-L-serine, SIGMA P–6641) was coated onto 96-well microplates (Poly soap Immuno Plate, NUNC) by evaporation. The plates were blocked Ca\(^2\)-Tris buffered saline (TBS : 10 mM TBS, PH 7.4–7.5) with 1% bovine serum albumin (BSA). The microplates were incubated for 120 minutes at room temperature and washed 2 times with Ca–TBS including 0.05% Tween 20. Then, 100 μl of serum samples or standard diluted 1 : 50 in BSA–Ca–TBS with human PT diluted 1 : 50 in Ca–TBS were added to the wells.

   The microplates were incubated for 90 minutes at room temperature and washed 3 times with Ca–TBS including 0.05% Tween 20. A peroxidase conjugated anti-human IgG or IgM antibody (Alkaline phosphatase-conjugated goat anti-human IgM and IgG, ZY laboratory) was added to each well and incubated for 1 hour at room temperature. After washing 3 times by Ca–TBS including 0.05% Tween 20, 100 μl of p–nitrophenylphosphate (ICN) was added and incubated for 10 minutes. The reaction discontinued by the addition of 3N NaOH. The optical density (OD) of each well was read at 405 nm using a microplate reader (BIO–RAD Model 550). As standards of these measurements, 2 kinds (IgG and IgM) of high-titer serum obtained from patients with APS were used. The standard curves for the aPS/PTAb (IgG and IgM) were proportional in the dilution of standards from 50 times to 12800 times (IgM) or from 800 times to 51200 times (IgG) respectively. The titers were set on 1000 U/ml for 50 times dilution of IgG and IgM. The cut off OD values (mean±3SD) were determined as : 4.9 for aPS/PTAb–IgG and 13.3 for aPS/PTAb–IgM in healthy nonpregnant women and as 13.2 for aPS/PTAb–IgG and 7.3 for aPS/PTAb–IgM in normal pregnant women.

3. **Statistical Analysis**

   Titters between groups were compared with unpaired t-test. Positive rates for 2×2 tables were compared using Fisher’s exact test. A P value<0.05 (two sided) was considered statistically significant.

**Results**

1. **aPS/PTAb in recurrent abortion cases.** (Fig. 1, Table I)

   The Values (mean±SD) of aPS/PT IgG and IgM antibody in non-pregnant women (n=16) were 2.07±0.95, 8.05±1.76, respectively. The cut off value were decided as 4.9 for IgG antibody and 13.3 for IgM antibody. In aPS/PTAb–IgG antibody measurements, 3 out of 86 cases (3.4%) were positive for aPS/PTAb. In aPS/PTAb–IgM antibody measurements,
5 out of 86 (5.8%) were positive for aPS/PTAb. The incidence of total aPS/PTAb in recurrent abortion cases was higher than that of 47 non-pregnant women (p=0.055).

2. The relationship between aPS/PTAb and anti β2-GPI antibody.

When the relationship between aPS/PTAb and anti β2-GPI antibody was evaluated, only one of 3 positive aPS/PTAb was positive for anti β2-GPI antibody.

3. The frequency of aPS/PTAb in early and late fetal abortion.

When the frequency of aPS/PTAb in the early or late abortion was evaluated, 6 out of 74 cases (8.1%) were positive for aPS/PTAb in recurrent embryonic losses at early fetal period (<10 weeks’ gestation) and 2 out of 12 cases (16.6%) were positive for aPS/PTAb in recurrent abortion at late fetal period (>10 weeks’ gestation).

No significant difference was found between early and late fetal abortion (p=0.40).

4. aPS/PTAb in preeclampsia cases. (Fig. 2, Table II)

The Values (mean±SD) of aPS/PT IgG and IgM antibody in normal pregnant women (n=16) were 6.56±2.21, 2.99±1.43, respectively. The cut off value were decided as 13.2 for IgG antibody and 7.3 for IgM antibody. In aPS/PTAb–IgG antibody measurements, 2 out of 82 cases (2.5%) were positive for aPS/PTAb. In aPS/PTAb–IgM antibody measurements, 13 out of 82 (16%) were positive for aPS/PTAb. The incidence of IgM or total aPS/PTAb in preeclampsia cases was higher than that of 40 normal pregnant women (p=0.005, p=0.002).

5. Values and positive rates of aPS/PTAb in severe or mild preeclampsia cases (Table III)

The values (mean±3SD) and positive rates of aPS/PTAb in patients with severe preeclampsia (9.9 ± 24.2, 21.5%) was higher tendency than in patients with mild preeclampsia (5.1 ± 1.7, 6.4%, p=0.117).

6. Values and positive rates of aPS/PTAb in preeclampsia cases with or without proteinuria (Table IV)

The values (mean±3SD) and positive rates of aPS/PTAb in proteinuria-positive cases (6.2 ± 3.8, 16.9%) was higher tendency than in patients with proteinuria-negative cases (5.0 ± 1.5, 4.3%, p=0.098).

7. Values and positive rates of aPS/PTAb in preeclampsia cases with or without hypertension (Table V)

The values (mean±3SD) and positive rates of aPS/PTAb in hypertension-positive cases (8.3 ± 19.9,21.0%) was higher tendency than in patients with hypertension-negative cases (5.1 ± 1.8, 4.0%, p=0.096).

8. Values and positive rates of aPS/PTAb in
Table IV  Values (mean and standard deviation) and positive rates of anti–phosphatidylserine–prothrombin antibody in preeclampsia cases with or without proteinuria

<table>
<thead>
<tr>
<th>Proteinuria</th>
<th>values</th>
<th>positive rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>standard deviation</td>
</tr>
<tr>
<td>positive</td>
<td>6.2</td>
<td>3.8</td>
</tr>
<tr>
<td>negative</td>
<td>5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*p = 0.06 **p = 0.098

*p-t-test, ** Fisher’s exact test

Table V  Values (mean and standard deviation) and positive rates of anti–phosphatidylserine–prothrombin antibody in preeclampsia cases with or without hypertension

<table>
<thead>
<tr>
<th>Hypertension</th>
<th>values</th>
<th>positive rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SBP &gt; 140 mmHg or DBP &gt; 90 mmHg</td>
<td>8.3</td>
<td>19.9</td>
</tr>
<tr>
<td>SBP &lt; 140 mmHg and DBP &lt; 90 mmHg</td>
<td>5.1</td>
<td>1.87</td>
</tr>
</tbody>
</table>

*p = 0.20 **p = 0.096

*p-t-test, ** Fisher’s exact test, SBP: systolic blood pressure, DBP: diastolic blood pressure

Table VI  Values (mean and standard deviation) and positive rates of anti–phosphatidylserine–prothrombin antibody in preeclampsia cases with severe hypertension or without hypertension

<table>
<thead>
<tr>
<th>Hypertension</th>
<th>values</th>
<th>positive rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SBP &gt; 160 mmHg or DBP &gt; 110 mmHg</td>
<td>10.1</td>
<td>25</td>
</tr>
<tr>
<td>SBP &lt; 140 mmHg and DBP &lt; 90 mmHg</td>
<td>5.1</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*p = 0.19 **p = 0.045

*p-t-test, ** Fisher’s exact test, SBP: systolic blood pressure, DBP: diastolic blood pressure

preeclampsia cases with severe hypertension (blood pressure greater than 160 mmHg systolic or 110 mmHg diastolic) or without hypertension (Table VI)

The Values (mean ± 3SD) and positive rates of aPS/PTAb in severe hypertension-positive cases (10.1 ± 25, 23.0%) was higher than in hypertension-negative cases (5.1 ± 1.9, 4.0%, p = 0.045). The value was statistically significant.

9. Values and positive rates of aPS/PTAb in preeclampsia cases with or without IUGR (Table VII, Table VIII)

The evaluation of IUGR was done by Nishida’s definition. Incidence of IUGR in all cases of preeclampsia was 35.3%. There were no significant difference between preeclampsia with and without IUGR concerning to age, gravity and mean of gestational week. Mean of neonatal body weight in preeclampsia cases without IUGR (2302 ± 794) was greater than in preeclampsia cases with IUGR (1696 ± 570) (p = 0.002). These incidence in aPS/PTAb positive and aPS/PTAb negative cases were 6.8% and 24.5%, respectively (p = 0.072). No significant relationship was found in positive rate of aPS/PTAb between preeclampsia with and without IUGR.

Discussion

Our results indicated that the both IgG and IgM types of aPS/PTAb were found in patients with recurrent abortion and preeclampsia, which were not related to anti-β2-GPI antibodies, gestational weeks of abortion, severity of preeclampsia, occurrence of IUGR, but related to only severe hypertension in preeclampsia patients. This may be related to the limited number of sample size, but the relation between aPS/PTAb and severe hypertension is very interesting.

Many kinds of methods to detect the antiprothrombin antibodies have been developed. In our study, a modification of the ELISA described by Matsuda was used to detect aPS/PTAb. We found the presence of IgG or IgM aPS/PTAb in patients with recurrent abortion. Incidence of aPS/PTAb—IgG (3.4%) was lower than those of anticardiolipin antibody (10.4%)
and antiphosphatidylserine antibody (4.6%) and β2-GPI antibody (4.6%) in our laboratory. Tsutsumi could not identify aPS/PTAb in a population of patients with unexplained miscarriages. They studied 86 consecutive patients with history of 2 or more recurrent miscarriages. However, patients with history of overt thrombotic events were not included. The titer of IgG, IgM and IgA aPS of overt thrombotic events were not included. The recurrent miscarriages. However, patients with history of recurrent miscarriages. In our study, when the cut-off levels were set at mean + 5SD, established using sera from 36 healthy volunteers. In systemic autoimmune disease by Atsumi. It has been reported that there is a different pathological mechanism between the early and late abortion. In anti-phosphatidylethanolamine antibody (aPEAb) positive cases, higher frequency of the early abortion has reported. When the frequency of the early and late abortion were evaluated, no significant difference was found between early and late fetal abortion. We think that A aPS/PTAb is a different type of antibody from aPEAb.

Akimoto et al. examined the levels of IgG autoantibodies against prothrombin in pregnant women. Ten (36%) of the 28 women with severe preeclampsia were positive for antiprothrombin antibodies as compared with 3 (9%) of the 36 women with normal pregnancies. In our cases, 11 (21.5%) of the 51 women with severe preeclampsia were positive. Our positive rate was lower than that of them. It may depend on methodological difference to detect antibodies against prothrombin. Our data shows that aPS/PTAb is a different type of antibody from aPSAb. Our results suggest that there may be a specific relation between aPS/PTAb and preeclampsia.

We have reported the presence of various kinds of antiphospholipid antibody in preeclampsia. The prevalence of anticardiolipin and antiphosphatidylserine antibody (aPSAb) were 14.7% and 18.1%, respectively. Though aPS/PTAb is a different antibody from aPSAb, the positivity (18.2%) of aPS/PTAb was same as that of aPSAb. It may be depend on the difference of method. In previous assay, Loizou method was used. However, the prevalence of IgG anticardiolipin, aPSAb and anti β2-GPI antibody were 10.0%, 18.1%, and 18.4%, respectively. The value (2.5%) of aPS/PTAb–IgG was lower than those of the 3 antibodies. The prevalence of IgM anticardiolipin and antiphosphatidylserine were 14.7% and 0%, respectively. The positivity (16.0%) of aPS/PTAb–IgM was higher than those of the 2 antibodies. Our finding seems to show that aPS/PTAb is a different type of antibody from these antibodies including aPSAb.

We analyzed the relationships between clinical finding of preeclampsia and aPS/PTAb. The prevalence of aPS/PTAb in severe cases was much higher than that of mild cases. In cases of severe hypertension, significant higher prevalence was found as compared with normal pregnancies. We have already reported
the presence of antiphosphatidylserine antibody (aPSAb) in preeclampsia. A aPSAb positivity in severe cases (22.7%) was greater than that in mild cases (9.1%).10 A aPSAb/PTAb seems to be much more related to severity of preeclampsia and hypertension.

The presence of IUGR in antiphospholipid antibodies has been reported.10,22 In this study, there was no relationship between aPS/PTAb positivity and IUGR. In our previous study, the incidence of IUGR in IgG anticardiolipin antibody (ACA) positive, IgM ACA positive and IgG aPSAb positive cases was 43%, 40%, and 50%, respectively. Sletnes et al. reported that the frequency of IUGR in anticephalin antibody positive women (57.1%) was significantly higher compared with the negative.23 In anti β2-GPI antibodies, that of IUGR was 43%. The incidences in positive cases were higher than that in negative cases. A aPS/PTAb dose not seem to relate the IUGR of preeclampsia. The pathogenic ability of aPS/PTAb may be different from ACA, aPSab and anti β2-GPI antibodies.

Coagulation changes such as increased levels of the thrombin anti-thrombin III complex (TAT) and the plasminogen inhibitor complex (PIC) in preeclampsia have been demonstrated.24 These changes in preeclampsia might be related to these antibodies. We have already reported the relationship between the anti-cardiolipin antibody and prolonged activated partial thromboplastin time (APTT) and low platelet counts.10 In our study, TAT was measured in 3 cases of aPS/PTAb positives. All cases showed high values such as 25.1, 12.0 and 8.2 ng/ml. We speculate that aPS/PTAb may play a role of coagulation changes in preeclampsia.

We concluded that aPS/PTAb appears in some cases of recurrent abortion and preeclampsia. Our data suggest that aPS/PTAb might be a risk factor in patients with recurrent abortion, and might relate to clinical finding in preeclampsia.

Acknowledgments: We thanks to Matsuda. J (Teikyo university school of medicine, Tokyo, Japan) for technical assistance of aPS/PT antibody, ELISA.

Reference


17) Papoian, R., et al.: Immunological regulation of spontaneous antibodies to DNA and RNA. II. Sequential switch from IgM to IgG in NZB/NZW F1 mice. Immunology. 32: 75–79, 1977


