Complement as a predictor of further miscarriage in couples with recurrent miscarriages

Mayumi Sugiura-Ogasawara¹, Kyoko Nozawa, Tamao Nakanishi, Yukio Hattori and Yasuhiro Ozaki

Department of Obstetrics and Gynecology, Nagoya City University Medical School, Nagoya, Japan

¹To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Nagoya City University Medical School, Mizuho-ku, Nagoya 4678601, Japan. E-mail: og.mym@med.nagoya-cu.ac.jp

BACKGROUND: The clinical significance of complement is unclear in patients with unexplained recurrent miscarriage, though low levels of complement 3 (C3) and/or complement 4 (C4) are reported to be associated with the antiphospholipid syndrome (aPS). We therefore investigated whether C3 and C4 have a predictive value for subsequent miscarriages. METHODS: In total, 215 patients with a history of two consecutive first-trimester miscarriages and no abnormal chromosomes in either partner, no uterine anomalies and no antiphospholipid (aPL) antibodies were examined. Blood tests for C3, C4, total haemolytic complement (CH50), immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were performed before subsequent pregnancy. Patients were then followed up without treatment, and their pregnancy outcomes were compared with their previous blood test results. RESULTS: From 215 pregnant patients, 45 subsequently miscarried, whereas the remainder had a live birth. There was no relation with serum CH50, IgG, IgA and IgM levels, but C3 and C4 levels in patients with subsequent miscarriage were significantly higher than in those whose pregnancy was successful. CONCLUSION: In patients with two previous miscarriages, C3 and C4 levels were higher in those women who had a third miscarriage, than in women that went on to have a live birth.

Key words: CH50/complement/IgA/IgG/IgM/recurrent miscarriage

Introduction

Established causes of recurrent miscarriages are chromosomal abnormalities in either partner, particularly translocations, as well as antiphospholipid (aPL) antibodies and uterine anomalies (Farquharson et al., 1984; Raga et al., 1997; Sugiura-Ogasawara et al., 2004). However, half of all cases are unexplained (Herz-Picciotto and Samuels, 1998), and thrombophilia and many kinds of polymorphisms have recently been reported to be associated with recurrent miscarriages (Nelen et al., 1996; Preston et al., 1996; Rey et al., 2003). It is well known that heritable thrombophilia is associated with fetal loss. We have found that shortened activated partial thromboplastin time (PTT), reduced coagulation factor XII activity and elevated natural killer (NK) cell activity may be predictors for further miscarriages in patients with recurrent miscarriages (Aoki et al., 1995; Ogasawara et al., 1998, 2001). We have also reported that anti-nuclear antibodies do not predict subsequent miscarriage, although they have been speculated to constitute a risk factor (Ogasawara et al., 1996a).

Complement is reported to be associated with the antiphospholipid syndrome (aPS) (Cowchock et al., 1984; Unander et al., 1987; Holers et al., 2002; Girardi et al., 2004). Recently, Girardi et al. presented evidence that complement 3 (C3) activation is required for aPL-induced pregnancy loss and that heparin prevents pregnancy loss by inhibiting complement activation. However, there is limited information about association between complement and unexplained recurrent miscarriage without the aPS. We therefore investigated whether levels of this factor might predict subsequent early miscarriage in patients with a history of first-trimester spontaneous abortion.

Subjects and methods

Patients

We here studied patients with a history of two consecutive first-trimester recurrent miscarriages. All patients were seen at Nagoya City University Hospital and underwent hysterosalphingography, chromosome analysis for both partners, immunologic tests for parameters such as NK cell activity and aPL and blood tests for hyperthyroidism, diabetes mellitus, hyperprolactinaemia and infections such as chlamydia, before subsequent pregnancy. On the basis of the results, patients with translocations in either partner or uterine anomalies such as uterus unicornis or bicornis were excluded. Patients with at least one kind of aPL, with testing on separate occasions, were also excluded from the analysis. We focused on only two consecutive miscarriages because we usually offer particular treatment for those with three or more consecutive miscarriages and cases with non-consecutive miscarriages are not examined clinically.

To detect β₂-glycoprotein I-dependent anticardiolipin antibodies, a modified ELISA system was applied (Matsuura et al., 1994). Lupus
Inflammatory parameters in patients with recurrent miscarriages

Anticoagulant was detected by diluted aPTT methods (Ogasawara et al., 1996b).

Blood tests for C3, complement 4 (C4), total haemolytic complement (CH50), total serum immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were performed at the same time before pregnancy.

A total of 215 patients again became pregnant, and all were admitted to Nagoya City University Hospital for rest for ~1 month at 4 weeks’ gestation to avoid possible external risk factors. The duration between examination and pregnancy was 7.3 ± 6.7 months. Ultrasonography was performed twice a week during pregnancy. When miscarriages were diagnosed, dilation and curettage (D & C) was performed, and the chromosomal karyotype of each aborted conceptus was examined. Informed consent, approved by the Institutional Review Board, was obtained from all patients.

The subsequent pregnancy outcome was followed up prospectively. Preconceptional values for C3, C4, CH50, IgG, IgA and IgM were compared between subsequent successful patients and miscarriage cases. Also, the values for each parameter were compared after excluding miscarriage cases caused by an abnormal embryonic karyotype.

In a separate retrospective study, we also investigated 12 patients with aPS defined by new International classification criteria (Miyakis et al., 2006). Two of them suffered from systemic lupus erythematosus (SLE) and two from mixed connective tissue disease. Eight of them were patients with primary aPS. Serum C3, C4, CH50, IgG, IgA and IgM levels were compared between 12 patients with aPS and 215 recurrent aborters without aPS.

**Complement 3 and 4, CH50, IgG, IgA and IgM**

Complement components C3 and C4 were determined by a nephrometric approach, with normal ranging from 45 to 95 and 12 to 40 mg/dl, respectively.

The normal range for CH50, determined by the Mayer method, was 29–48 U/ml.

Normal ranges for IgG, IgA and IgM were 921–1868, 113–402 and 61–277 mg/dl, respectively, also determined by nephrometry.

**Statistical analysis**

Data were analysed by the Student’s t-test using Stat View 4.0 and an Apple Macintosh computer. A significance level of P < 0.05 was applied for all tests.

**Results**

A total of 215 patients became pregnant subsequently. Of the total, 45 (20.9%) miscarried again. An abnormal embryonic karyotype was found in 8 of 17 cases analysed (47.1%). The mean ± SD for age of all patients was 29.1 ± 3.8.

Mean (±SD) values for preconception C3 in patients with subsequent reproductive success and failure were 67.4 ± 17.5 and 74.6 ± 20.6 mg/dl, respectively (P = 0.020; Table I). Mean (±SD) values for preconception C4 in patients with subsequent reproductive success and failure were 22.3 ± 7.9 and 25.6 ± 8.7 mg/dl, respectively (P = 0.016).

When eight miscarriage cases caused by an abnormal embryonic karyotype were excluded, mean (±SD) values for preconception C3 and C4 in patients with subsequent failure were 76.2 ± 22.0 and 25.8 ± 8.6 mg/dl, respectively. The difference of C3 and C4 from the successful cases remained significant (P = 0.0087 and P = 0.018, respectively). In contrast, there were no significant differences in mean values for preconception IgG, IgA, IgM and CH50 between groups with subsequent success and failure (Table I).

Twelve of 31 patients (38.7%) with elevated levels of C3 and 33 of 184 patients (17.9%) with normal values miscarried again when the mean +1SD value of C3 (84.9 mg/dl) for successful pregnancies was defined as the threshold point. Thirteen of 37 patients (35.1%) with elevated C4 and 32 of 178 patients (18.0%) with normal values miscarried again when the mean +1SD value of C4 (30.2 mg/dl) for successful pregnancy was defined as the threshold point. Elevated levels of C3 and C4 predicted subsequent miscarriage significantly (P = 0.017 and P = 0.035, respectively).

Mean (±SD) values for all parameters in 12 aPL-positive patients and 215 patients without aPL are summarized in Table II. C3, C4, IgM and CH50 levels in aPL-positive patients were significantly lower than those in patients without aPL.

**Discussion**

In the present study, elevated levels of C3 and C4 were found to predict subsequent miscarriage in patients suffering from unexplained recurrent miscarriages. To our knowledge, there have been no earlier reports concerning such an association. There were no significant differences in mean values for preconception IgG, IgA, IgM and CH50 between groups with subsequent success and failure. It is well established that IgG, IgA and IgM autoantibodies to phospholipids are associated with

**Table I. Inflammatory parameters in patients with recurrent miscarriages**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pregnancy outcome</th>
<th>Live birth (n = 170)</th>
<th>Miscarriage (n = 45)</th>
<th>P-value</th>
<th>Miscarriage excluding abnormal embryonic karyotype (n = 37)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td></td>
<td>28.9 ± 3.7</td>
<td>29.2 ± 4.2</td>
<td>NS</td>
<td>76.2 ± 22.0</td>
<td>0.0087</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td></td>
<td>67.4 ± 17.5</td>
<td>74.6 ± 20.6</td>
<td>0.020</td>
<td>25.8 ± 8.6</td>
<td>0.018</td>
</tr>
<tr>
<td>C4 (mg/dl)</td>
<td></td>
<td>22.3 ± 7.9</td>
<td>25.6 ± 8.7</td>
<td>0.016</td>
<td>255.6 ± 72.2</td>
<td>NS</td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td></td>
<td>1608.0 ± 309.3</td>
<td>1578.3 ± 273.5</td>
<td>NS</td>
<td>230.4 ± 99.4</td>
<td>NS</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td></td>
<td>261.0 ± 96.1</td>
<td>262.7 ± 69.3</td>
<td>NS</td>
<td>35.8 ± 5.9</td>
<td>NS</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td></td>
<td>223.3 ± 84.0</td>
<td>242.6 ± 99.3</td>
<td>NS</td>
<td>35.8 ± 5.9</td>
<td>NS</td>
</tr>
<tr>
<td>CH50 (U/ml)</td>
<td></td>
<td>34.7 ± 6.5</td>
<td>35.6 ± 5.5</td>
<td>NS</td>
<td>35.8 ± 5.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

C3, complement 3; C4, complement 4; CH50, total haemolytic complement; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.
recurrent miscarriages. However, to our knowledge, any association between non-specific total serum IgG, IgA and IgM and recurrent miscarriage in patients without autoimmune disease remains unclear.

It is well known that C3 and C4 levels are low in patients with SLE. In previous studies, C3 and C4 levels were found to be lower in recurrent miscarriage with autoantibodies without SLE. Unander et al. (1987) reported significantly low levels of C4 in primary habitual aborters with high anticardiolipin antibody values. Cowchock et al. (1984) found C3 and C4 levels to be significantly lower in patients with unexplained habitual abortion and subclinical autoimmune diseases than in cases with explained habitual abortion. In the present study, C3 and C4 levels in patients with aPS were also significantly lower than those in patients without aPL.

aPS is defined by thrombosis and recurrent pregnancy loss in the presence of aPL and is generally treated with combined low-dose aspirin and heparin therapy. Many kinds of mechanisms by which aPL could cause pregnancy losses have been proposed. For example, Rand et al. (1997) reported reduction in the levels of annexin V, a placental anticoagulant protein, and accelerated coagulation of plasma with cultured trophoblasts and endothelial cells. We found that β₂-glycoprotein I has inhibitory effects on coagulation factor X and that β₂-glycoprotein I-dependent anticardiolipin antibodies prevent the inhibitory effects of β₂-glycoprotein I on factor X and induce uteroplacental blood insufficiency (Ogasawara et al., 1995). It is generally speculated that aPL causes pregnancy loss through the insufficiency in blood supply resulting from uteroplacental thrombosis. Indeed, thrombophilia is associated with pregnancy loss by a mechanism featuring thrombosis in the placenta, which causes intrauterine fetal death or growth retardation in some way (Preston et al., 1996; Rey et al., 2003).

Recently, mechanisms by which aPL could cause pregnancy loss without thrombosis have been suggested. Quenby et al. (2005) found that aPL can inhibit extravillous trophoblast differentiation into giant multinucleated cells in vitro, and a failure of subsequent uteroplacental development may be an underlying pathology in aPL-associated pregnancy loss. Bose et al. (2004) concluded that lupus anticoagulant may impair placentation by increasing apoptosis, attenuating mitosis and reducing invasion of the trophoblast.

Regarding complement, Holers et al. (2002) found that its activation is essential and causative in aPL antibody-induced fetal injury, and Girardi et al. (2004) reported that C3 activation is required for aPL-induced pregnancy loss, with heparin exerting preventive effects by inhibiting complement activation. They provided evidence that unfractionated heparin and low molecular weight heparin but not fondaparinux and hirudin can inhibit C3 activation by aPL-IgG in mice and in vitro.

The present study indicates that complement activation also might be causative in aPL-negative recurrent aborters. C3 and C4 are well known to be elevated with inflammation. After implantation the trophoblast rapidly invades the decidua and spiral artery, where a loose plug of endovascular trophoblast cells forms and receives blood flow. It is necessary to maintain this slow blood flow to supply the trophoblast itself, which releases both pro-inflammatory and anti-inflammatory cytokines to regulate the balance in the fetal–maternal interface (Wegmann et al., 1993). A T-helper 1 (Th1) shift has been speculated to cause recurrent miscarriage. However, Th1/Th2 failed to predict subsequent miscarriage in patients with recurrent miscarriages (Shimada et al., 2004). Bacterial vaginosis is reported to be associated with sporadic abortion (Leitich et al., 2003) but has not shown any link to recurrent miscarriages, and we found no influence for Chlamydia trachomatis (Sugiura-Ogasawara et al., 2005). In fact, no inflammatory parameters have been shown to predict subsequent miscarriage.

Heparin has also been reported to prevent miscarriage in patients not only with aPL but also in some cases with unexplained recurrent miscarriage, though it has not been established that heparin is effective for unexplained recurrent miscarriages (Miyashita et al., 2003). Elevated C3 and/or C4 might be candidate predictors for those in which heparin might be most useful.

This must be considered a preliminary study because the sample size was relatively small. However, given the paucity of predictors for miscarriage other than aPL, chromosome abnormalities in either partner, abnormal embryonic karyotype and maternal age, as well as the limited numbers of recurrent aborters who can presently be successfully treated with medication, our present findings may be of major significance and warrant further attention.

References


Submitted on March 13, 2006; resubmitted on April 17, 2006, May 12, 2006; accepted on May 15, 2006.