Original Article

Antiphospholipid Antibody Titers and Clinical Outcomes in Patients with Recurrent Miscarriage and Antiphospholipid Antibody Syndrome: A Prospective Study

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Abstract

Background: The management of patients with recurrent miscarriage (RM) and antiphospholipid antibody syndrome (APS) includes prolonged treatment with heparin and aspirin, starting from the confirmation of pregnancy and continuing until 6 weeks after birth. This study was conducted to determine the relationship between changes in antiphospholipid antibody titers and clinical outcomes. The effect of a shortened treatment regimen was also evaluated.

Methods: A prospective study of 123 patients with RM and APS between March 2012 and May 2014 was conducted. Patients were pretreated with a low dose of prednisone plus aspirin before pregnancy, and heparin was added after conception. The levels of antiphospholipid antibodies and pregnancy outcomes were evaluated.

Results: All patients were positive for anti-β2-glycoprotein 1 (anti-β2-GP1) IgM. After prepregnancy treatment with low-dose prednisone plus aspirin, 99 of 123 patients became pregnant, and 87 of those pregnancies resulted in successful live births, while 12 resulted in miscarriage, showing a success rate of 87.9%. In the live birth group, levels of anti-β2-GP1 were 56.8 ± 49.0 RU/ml before the pretreatment regimen, 32.1 ± 26.0 RU/ml after 2 months of pretreatment, and 24.1 ± 23.1 RU/ml during early pregnancy ($P < 0.05$). In the miscarriage group, antiphospholipid antibody titers were 52.8 ± 30.7 RU/ml before pretreatment, 38.5 ± 34.2 RU/ml after pretreatment, and 33.9 ± 24.7 RU/ml during early pregnancy; the decrease in antiphospholipid antibodies was lower in the miscarriage group than in the live birth group ($P < 0.05$). Of the 24 infertile patients, the average antibody titer did not decline after pretreatment ($P = 0.802$).

Conclusions: Anti-β2-GP1 IgM was the predominant form of antibody in patients with RM and APS. The decreases in antiphospholipid antibody titers correlated with better pregnancy outcomes. The shorter treatment regimen was effective and economical.

Key words: Anticoagulant Therapy; Antiphospholipid Antibody; Antiphospholipid Antibody Syndrome; Recurrent Miscarriage

Introduction

Antiphospholipid antibody syndrome (APS) is a noninflammatory autoimmune disease characterized by the generation of antiphospholipid antibodies. APS can result in thrombosis and pregnancy loss.[1] Women are more likely to suffer from the disease than men.[2] Women of childbearing age are often first diagnosed with APS when they seek medical treatment for recurrent pregnancy loss. At present, recurrent miscarriage (RM) is generally defined as the loss of two or more pregnancies with the same partner before the first 24 weeks of gestation. While the cause of RM is indeterminable in 50% of patients, APS is one of the main causes of miscarriages in 15% of patients.[3] Most women suffering from RM require curettage treatment, which can lead to infertility and have a serious impact on women's physical and mental health. Even if patients with both APS and a history of RM do become pregnant, they face a risk of serious obstetric complications such as pre-eclampsia and HELLP syndrome (hemolysis, elevated liver enzymes,

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and low platelets), which can be life-threatening to both mother and the fetus. The etiology of APS is far-ranging, and the exact pathogenesis of the disease is not yet clear. At present, etiological research has focused mainly on the release of inflammatory cytokines, enhanced platelet aggregation, coagulation abnormalities, and trophoblast cell damage, all of which are induced by the interactions between antiphospholipid antibodies and trophoblast cells, endothelial cells, and lymphocytes.[4] Wang *et al*.[5] studied the placental anatomy of miscarriage patients with APS and found diffuse thrombosis in the uterine spiral artery and other small vessels. Based on these findings, anticoagulation is regarded as the core treatment for APS patients. Common medications include prednisone, aspirin, and heparin. Combinations of these drugs are considered better than single-drug treatments,[6] and the live birth rate for pregnant patients undergoing this treatment is 73–79%.[7] However, it is recommended that medication should be administered from the time pregnancy is confirmed until 6 weeks postpartum.[8] So far, the relationship between antiphospholipid antibody titers during treatment and pregnancy outcomes has not been described in detail. Therefore, as a treatment strategy for APS patients with RM, we prospectively prescribed prednisone and aspirin before pregnancy and low-molecular-weight heparin (LMWH) once pregnancy was confirmed. The isotype of antiphospholipid antibodies, changes in antibody titers during treatment, time of medicine discontinuance, reported side effects, and pregnancy outcomes were evaluated to analyze the relationship between changes in antibody and clinical outcomes. This study should provide a reference for the evaluation of therapeutic effects and the clinical treatment of patients with APS and RM.

Methods

Inclusion criteria

Patients who were diagnosed with APS and RM at the Reproductive Medicine Center of the Peking University Third Hospital (Beijing, China) between March 2012 and May 2014 were enrolled in this study (n = 123). Patients with abnormal uterine anatomy, endocrine abnormalities, or parental chromosome abnormalities were excluded. The clinical diagnosis was based on the standard from the Sapporo International Conference in 2006.[4] According to these criteria, at least one clinical and one laboratory test must be met for a diagnosis of APS. Due to the fact that our clinical laboratory can only measure the IgG and IgM isotypes of anticardiolipin and anti-β2-glycoprotein 1 (anti-β2-GP1) antibodies, these were used as the screening indexes for APS. This study was approved by the Institutional Review Board of Peking University Third Hospital and was conducted according to the tenets of the *Declaration of Helsinki* and its revisions.

Antiphospholipid antibody testing

Blood samples were sent to the clinical laboratory at Peking University Third Hospital and tested using an enzyme-linked immunosorbent assay kit from EUROIMMUN (Germany). The test kit included microplates, standard 1 (120PL-IgG/IgM-U/ml), standard 2 (12PL-IgG/IgM-U/ml), standard 3 (2PL-IgG/IgM-U/ml), negative and positive controls, peroxidase-conjugated rabbit anti-human IgG/IgM, sample buffer, wash buffer, and stop solution. Serum samples were diluted 1:20 in sample buffer. For quantitative detection, standards 1, 2, and 3 were incubated with positive control, negative control, or patient serum. The upper limit of detection for anticardiolipin antibodies is 12 PLU/ml, and the detection limit for anti-β2-GP1 antibodies is 20 RU/ml. The test kit has few interference factor and can support repeatable tests.

Methods of treatment

Patients chosen according to the inclusion criteria were pretreated with a low dose of prednisone (5 mg once a day [QD] by mouth [PO]) and aspirin (75 mg QD PO) for 2 months. During this period, patients were asked to use contraception. After pretreatment, the contraception was discontinued but the treatment was continued. Pregnancies were confirmed by the date of the last menstrual cycle and by changes in human serum chorionic gonadotropin (hCG) levels. LMWH (4100 international units QD, subcutaneously [SC]) was added to the regimen.

The antiphospholipid antibody titer was measured at the beginning and end of the 2-month pretreatment period and at about 6 weeks in early pregnancy. During pregnancy, the antiphospholipid antibody titer, platelet aggregation rate, and D-Dimer level were tested monthly to adjust the drug dosage, while symptoms such as vaginal bleeding were monitored. If the antibody test was negative two times in a row, all medication was stopped. Moreover, if patients showed obvious bleeding, aspirin and LMWH were stopped or the dosage was reduced and the pregnancy was strictly monitored. To avoid the side effects of hormone treatment, prednisone was used at most for 30 weeks during pregnancy.

In patients whose pregnancies resulted in a live birth, information on the newborn, including gender, birth weight, and fetal development, was recorded. Pregnancy-related complications were also analyzed in the live birth group, including preeclampsia, gestational diabetes, premature delivery (delivery between 28 and 37 weeks of pregnancy), premature rupture of membranes (spontaneous rupture of membranes before the onset of labor), and postpartum hemorrhage (bleeding reaches ≥500 ml for vaginal delivery and ≥1000ml for cesarean sections). Preeclampsia occurring after week 20 of pregnancy was defined as systolic blood pressure ≥140 mm Hg and/or diastolic pressure ≥90 mm Hg, with urinary protein ≥0.3 g per 24 h. Gestational diabetes mellitus (during 24–28 weeks of pregnancy) was diagnosed as blood glucose values of 5.1 mmol/L, 10.0 mmol/L, and 8.5 mmol/L before, 1 h after, and 2 h after the 75 g oral glucose tolerance test, respectively.[9,10] Side effects associated with the medication, such as allergy, serious bleeding, and subcutaneous ecchymosis, were also recorded.

Statistical analysis

All statistical analyses were done using SPSS 16.0 (SPSS

Inc., USA). The major statistical tests applied were the independent *t*-test, rank test, Chi-square test, and repeated variance analysis. A value of $P < 0.05$ was considered statistically significant.

Results

Pregnancy outcome

Of 123 patients, 99 had successful pregnancies, of which 87 resulted in live births, for an overall success rate of 87.9% (87/99). There were 12 cases of miscarriage and 24 patients who did not become pregnant by the time the data were collected. Specifically, the live birth group included four cases of preterm labor (4%) and 83 cases of full-term birth (83.8%), while the miscarriage group included one case of biochemical pregnancy (1%) and 11 cases of embryo damage (11.1%). No stillbirths occurred. In 4 of the 11 cases of embryo damage, chorionic chromosome testing was performed, and three abnormal results were found: tetraploid karyotype, trisomy 18, and trisomy 16.

Antibody expression

All 123 patients were positive for anti-β2-GP1 IgM while 13 of the 123 patients (10.6%) were also positive for anticardiolipin IgM. Table 1 shows the antibody types detected in each of the patient groups.

Changes in antiphospholipid antibody titers during treatment

Age, body mass index (BMI), and antibody titers before pretreatment, after 2-month pretreatment, and during early pregnancy were analyzed using SPSS version 16.0 [Table 2]. Given that 100% of patients were positive for anti-β2-GP1 IgM, this antibody was used as the representative index for the analysis of antiphospholipid antibody titers. The 123 patients ranged in age from 34 to 42 years. There was no significant difference in age, BMI, or history of pregnancy loss between the two groups ($P > 0.05$). The time until pregnancy in the miscarriage group was longer than that in the live birth group ($P < 0.001$). The antibody titer before medication in the live birth group was higher than that in the miscarriage group ($P < 0.05$). After 2 months of pretreatment, the antibody titer had gradually decreased in both groups and was lower in the live birth group than in the miscarriage group ($P < 0.05$). The percentage of patients who were negative for antiphospholipid antibodies was slightly lower in the live birth group than in the miscarriage group ($P < 0.05$). In early pregnancy, the live birth group had a lower average antibody titer and a higher proportion of patients who were negative for antiphospholipid antibodies ($P < 0.05$).

Table 1: Anti-phospholipid antibody types

Type of antibody	Live-birth ($n = 87$)	Miscarriage ($n = 12$)	Infertility ($n = 24$)
Anti-β2-GP1 (IgM)	75	11	24
Anti-β2-GP1 (IgM) plus anti-cardiolipin (IgM)	12	1	0

Data are given as the number of patients in the treatment. The Live-birth group ($n = 87$): Received prednisone 5 mg + aspirin 75 mg + LWMH 4100IU daily; Miscarriage Group ($n = 12$) : Received prednisone 5 mg+aspirin 75 mg + LWMH 4100IU daily; Infertility Group ($n = 24$): Received prednisone 5 mg + aspirin 75 mg daily.

In the live birth group, titer values were analyzed at the three time points by the paired *t*-test. The results showed that there were significant differences between each of the two titer values ($P < 0.05$), indicating that the medication effectively downregulate the serum level of antiphospholipid antibody in the patients. In addition, using the same analysis, similar results were obtained in the miscarriage group. Furthermore, we found that, at each time point, the titer levels in the two groups were significantly different ($P < 0.001$). Figure 1 shows the change in the average antiphospholipid antibody titers in both miscarriage group and live birth group. The decrease in antiphospholipid antibodies was significantly lower in the miscarriage group than in the live birth group ($P < 0.001$).

In addition, there were 13 patients who were positive for anticardiolipin antibodies. Their average titer before treatment was 38.1 ± 29.0 PLU/ml. After 2 months of pretreatment, each of the patients tested negative, and no recurrences were detected.

With respect to the 24 patients who were not pregnant at the time the data were analyzed, the average antibody titer before pretreatment was 55.0 ± 52.0 RU/ml, and no significant differences in antibody titer were found after 2 months of pretreatment (56.6 ± 32.1 RU/ml; $P = 0.802$).

Medication cutoff

In the live birth group, low-dose prednisone was stopped at 9–30 weeks of pregnancy (average, 17.9 ± 4.7 weeks), and aspirin was stopped at 10–31 weeks (average, 19.2 ± 5.0 weeks). Moreover, LMWH was stopped at 9–36 weeks (average, 22.5 ± 6.9 weeks).

Neonatal outcomes and side effects

During the treatment, 87 live births occurred. Among the newborns, there were 44 males and 43 females, for a sex ratio of 1.02:1. Of the 87 live births, 83 were full-term pregnancies, and the average birth weight was 3314 ± 200 g. The remaining four infants were born preterm as follows: male, 32 weeks, 2300 g; male, 34 weeks, 2705 g; male, 36 weeks, 2960 g; and female, 36 weeks, 2650 g. Follow-up interviews were conducted by phone, and no obvious developmental abnormalities in these newborns were revealed.

With respect to pregnancy complications, three cases of preeclampsia, one case of gestational diabetes mellitus, and six cases of premature rupture of membranes occurred in the 87 live birth patients.

Based on analyses of medical records and the telephone follow-up interviews, no postpartum bleeding or obvious medical side effects occurred.

Table 2: Changes in anti-β2-GP1 antibodies under medication

Items	Live-birth (n = 87)	Miscarriage (n = 12)	Statistical values	P
Age (years)	32.8 ± 4.3	30.8 ± 5.1	−0.194*	0.902
Body mass index (kg/m^2)	22.3 ± 2.9	22.7 ± 1.7	−0.576*	0.620
History of pregnancy loss (times)	2.3 ± 2.9	2.4 ± 0.7	−0.537*	0.710
Titer before medication (RU/ml)	56.8 ± 49.0	52.8 ± 30.7	−2.857*	0.030
Titer after 2 months of medication (RU/ml)	32.1 ± 26.0	38.5 ± 34.2	−2.701*	0.040
Titer in early pregnancy (RU/ml)	24.1 ± 23.1	33.9 ± 24.7	−2.611*	0.009
Negative rate after pre-treatment (%)	43.7/(38/87)	50.0/(6/12)	5.858†	0.040
Time until pregnancy (Months)	4.0 ± 2.6	10.0 ± 8.4	−3.051*	0.001
Negative rate in early pregnancy (%,(n))	60.9 (53/87)	33.3 (4/12)	8.364†	0.016

Data are given as mean ± SD, or different value (%). SD: Standard deviation; *Wilcoxon rank sum test; †Chi-square test. Time spent until pregnancy: the number of months from the start of medication until the confirmation of pregnancy; The negative rate: the number of cases in which the antibody titer was negative out of the total number of cases in the group.

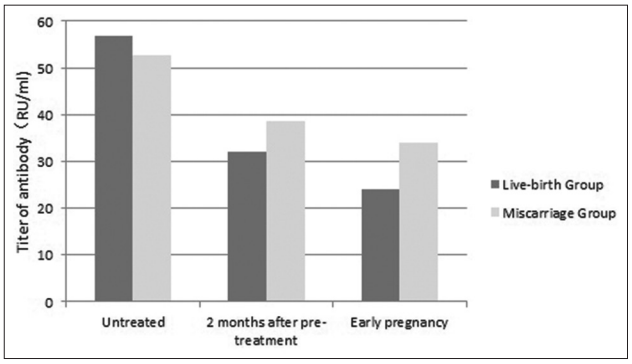

Figure 1: Change in the average antiphospholipid antibody titers in both miscarriage group and live birth group.

Discussion

Anti-β2-GP1 Ig-M antibodies were the predominant form of antiphospholipid antibody in patients with RM and APS in this study. This implies that β2-GP1 is the main antigen indicated in the disease pathology. In addition, it is the first time that we report a correlation between the change in antiphospholipid antibody titer during treatment and the pregnancy outcome. During the treatment process, we observed that the more the antibody titers decreased, the more likely patients were to experience a successful outcome. Moreover, in the present study, the treatment with low-dose prednisone combined with aspirin and LMWH resulted in a pregnancy success rate of 87.9% (87/99), which indicates that the shorter treatment regimen can improve the pregnancy outcome in an effective way.

Antigen-antibody recognition is a key to the immune response, and the main antigens in APS are cardiolipin and phospholipid acid, which are located on the cell membrane. Because lipids are weak in antigenicity, the production of antibodies requires proteins or carbohydrates as carriers. β2-GP1, which is synthesized by liver cells, is a protein normally found in the human plasma that inhibits the activation of coagulation factor X and XI, regulates the coagulation pathway, and reduces the occurrence of abnormal coagulation events. Recent research indicates that β2-GP1 acts as a cofactor for phospholipids to successfully trigger an immune reaction.[11] β2-GP1 plays an auxiliary role in the binding of antiphospholipid antibodies to their corresponding antigens. When β2-GP1 binds to negatively charged phospholipids on the cell membrane, the glycoproteins become phospholipid-protein binding complexes through conformational changes that expose the key sites to which antiphospholipid antibodies bind.[12] Another study revealed that β2-GP1 may also be the main antigen for anticardiolipin antibodies. In an *in vitro* experiment, researchers found that, without β2-GP1, anticardiolipin antibodies isolated from APS patients' serum bound to cardiolipin. However, when β2-GP1 was added, anticardiolipin antibodies had a higher affinity for β2-GP1 than for cardiolipin. During the process of zygote implantation, β2-GP1 combined with phospholipid on the trophocyte membrane; anti-β2-GP1 antibodies therefore damaged the trophocyte and caused pregnancy loss.[13,14] By retrospectively analyzing 152 healthy women, 141 women with recurrent spontaneous abortion (RSA) but without APS, 58 women with a history of fetal death, and 73 women with APS, Richard[15] reported that anti-β2-GP1 antibody titers in the APS patients were significantly higher than in the other three groups ($P < 0.001$). In addition, the percentage of APS patients with anti-β2-GP1 antibodies of the IgM isotype was higher than the percentage in the RSA group, fetal death group, and fertile control group, whereas there were no statistically significant differences in the percentage of anti-β2-GP1 antibodies of the IgG isotype between the four groups, which is in accordance with the results reported in this paper. When we reviewed the literature, we could not find reports of pathogenic phenotypes linked to the IgG and IgM types of anti-β2-GP1 antibody. Moreover, anti-β2-GP1 and anticardiolipin antibodies of the IgG type were rarely observed in our study.

As for the treatment regimen, before treatment, the antibody titer in the live birth group was higher than in the miscarriage group. During treatment, however, the titer in the patients who went on to experience successful pregnancies decreased more rapidly, and the time to conception was also shorter. In addition, in the treatment, no significant decrease in antibody titers was observed after treatment in the patients who did not conceive. Therefore, the odds of a successful pregnancy

were relatively low in patients who did not show an effective decrease in antibody titer after pretreatment. Out *et al*.[16] also reported that antiphospholipid antibody levels were closely related to adverse obstetric outcomes.

We found that the extent of the decrease in the antibody titer was associated with pregnancy outcomes. Pretreatment with prednisone helped lower the antibody titer. Because of its ability to inhibit antibody formation, prednisone has long been used in the treatment of RM and APS. The use of prednisone in APS patients with RM was first reported by Lubble in 1993, but the regimen was not recommended because of maternal complications caused by the high dose of the medication (40 mg daily).[7] Clinically, 5–10 mg of prednisone is administered once per day to avoid side effects. In this study, we only administered 5 mg of prednisone PO once per day, and the medication was continued for 17.9 ± 4.7 weeks into pregnancy. Pregnancy complications, neonatal abnormalities, and developmental abnormalities were uncommon. The main side effects of prednisone include obesity and liver dysfunction, which did not occur in our study. Recently, the British Society for Rheumatology reported that it is viable and safe to use prednisone during preconception and pregnancy, and even during lactation, because very little is secreted in the breast milk.[17] In China, Lin[6] recommended that the medication should be started as soon as pregnancy is confirmed and continued until 1–2 weeks after a negative antibody test. For patients whose antibody titers remain positive, prednisone should not be stopped. To avoid side effects of prednisone, we did not continue medication for more than 30 weeks into pregnancy, even if the antibody titer did not turn negative.

In the past 20 years, treatment for APS has gradually evolved from a single drug to combined therapy. Combining low-dose prednisone with aspirin and heparin improves pregnancy outcomes. In the present study, treatment with low-dose prednisone combined with aspirin and LMWH resulted in a pregnancy success rate of 87.9% (87/99). The pathology of APS is closely related to the formation of placental vascular thrombosis.[5] Because of the elevated coagulation status, patients with APS face a greater risk of thrombosis during pregnancy. By comparing the placenta of fetal growth restriction in patients with and without APS, Out *et al*.[18] reported a significantly higher rate of placental thrombosis in APS patients. In that study, of 16 APS patients, the authors found 13 cases of placental microcirculation thrombosis and/or placental infarct formation. Since 2000, researchers have gradually reached a consensus that the hypercoagulable state is a primary cause of adverse pregnancy events such as placental thrombosis. Heparin and aspirin are administered because of their effects on coagulation.[17] Besides its ability to inactivate coagulation factors, heparin can also block the ability of antiphospholipid antibodies to bind to trophoblast cells, thereby increasing trophoblast cell invasion and differentiation.[7] Furthermore, Di Simone *et al*. found that one mechanism for the pathogenesis of APS is hyperactivation of the complement system, a process that can be blocked by heparin.[17] Girardi *et al*.[19] confirmed that both heparin and LMWH can effectively block the activation of complement. Heparin was also found to inhibit the activation of the complement system and prevent pregnancy loss in mice with APS. The main side effects of heparin on patients are allergic reactions and bleeding, which were not seen during the present study. LMWH was discontinued at 22.5 ± 6.9 weeks of pregnancy and was not used during the postpartum period, which was not only effective but also economical.

Besides heparin, aspirin is also a main therapeutic for the treatment of APS and RM. By inhibiting platelet aggregation and decreasing the activity of prostaglandin synthase, aspirin can effectively reduce thrombosis. In a 1988 study, oral aspirin (QD 75 mg) was used exclusively to treat 42 APS patients with adverse pregnancy history. After the application of aspirin, the rate of live births improved significantly, from 10% before treatment to 88%.[20] Currently, RM with APS is generally treated with a combined regimen including aspirin. The most frequent side effects are peptic ulcers and bleeding, but no adverse events of aspirin therapy occurred in this study, and aspirin was stopped at 19.2 ± 5.0 weeks of pregnancy.

Kutteh[21] reported that 25 APS patients with RMs treated by oral aspirin (81 mg/d) before conception or subcutaneous injections of heparin (5000 units twice daily) to them once confirmed pregnancy at average of 5.3 ± 1.1 gestational weeks by tests of human chorionic gonadotropin with any missed period. Medication including heparin was continued until birth. The live birth rate was 80% (20 patients). For main gestational complications, the occurrence rates of diabetes mellitus, postpartum bleeding, and preeclampsia were 10%, 15%, and 10%, respectively. In our study, because aspirin and heparin were administered over a shortened period and only a half dosage of LMWH was administered via daily subcutaneous injection (compared with the dosage used in the previously mentioned study), no postpartum bleeding or obvious medical side effects occurred.

In conclusion, this is the first study in which low-dose prednisone and aspirin were used to pretreat RM patients with APS before pregnancy. Anti-β2-GP1 IgM was the predominant type of antiphospholipid antibody in these patients. A correlation was found between decreases in antiphospholipid antibody titers and better pregnancy outcomes. These results show that the shorter treatment regimen can effectively reduce antibody titers and improve pregnancy outcomes. The therapy was also safe and economical.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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