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High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies

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Antiphospholipid antibodies (APA), lupus anticoagulant (LA) and/or anticardiolipin antibodies (ACA), are associated with thrombosis and recurrent miscarriage. We studied the outcome of 20 pregnancies in women (median age 32 years; range 23–41) with APA (14 LA positive; three immunoglobulin (Ig) G ACA positive; two IgM ACA positive and one LA and IgG ACA positive) and history of recurrent miscarriage (median 4; range 3–11) who declined pharmacological treatment in their next pregnancy. Comparison was made with a cohort of 100 consecutive women (median age 33 years; range 23–44) with recurrent miscarriage (median 4; range 3–10), in whom no underlying cause to account for their pregnancy losses was found. Of the 20 women with APA, 18 (90%) miscarried compared to 34 of the 100 women (34%) with normal investigations ($P < 0.001$). The majority (94%) of miscarriages in women with APA occurred in the first trimester. Fetal heart activity was seen prior to fetal death in 86% of women with APA compared to 43% of women with normal investigations ($P < 0.01$). The first trimester loss of embryonic pregnancies is the most common type of miscarriage in women with APA. This may be a result of defective implantation and subsequent placentation.

Key words: antiphospholipid antibodies /pregnancy/prospective study/recurrent miscarriage

Introduction

Antiphospholipid antibodies (APA), the lupus anticoagulant (LA) and/or anticardiolipin antibodies (ACA), are a group of autoantibodies which are clinically important because of their strong association with recurrent miscarriage, thrombosis, and thrombocytopenia – the primary antiphospholipid syndrome (Harris, 1987).

Retrospective studies have reported a miscarriage rate of up to 90% in the previous pregnancies of women investigated for recurrent pregnancy loss who are found to have APA (Lubbe *et al.*, 1985; Branch, 1987). However, these retrospective studies fail to establish a temporal relationship between the presence of APA and pregnancy loss. Prospective data on the outcome of pregnancy in women with APA who received no pharmacological treatment, whilst sparse, suggest a fetal loss rate of between 50 and 75% (Lockwood *et al.*, 1989; Pattison *et al.*, 1993). These data are based on women with a 'low risk obstetric history' who have been studied from the time they attended their first antenatal clinic appointment, usually at the end of the first trimester of pregnancy. Hence, women with APA who miscarried in the first trimester of pregnancy have been excluded from these studies.

The high retrospective rate of fetal loss reported in women with APA has led to the use of aspirin (Lockshin *et al.*, 1989; Sanchez Guerrero and Alarcon Segovia, 1992; Silver *et al.*, 1993), heparin (Rosove *et al.*, 1990; Cowchock *et al.*, 1992), immunoglobulin (Carreras *et al.*, 1988; Scott *et al.*, 1988; Parke *et al.*, 1989) and steroids (Lubbe *et al.*, 1983; Lockshin *et al.*, 1989; Cowchock *et al.*, 1992; Silver *et al.*, 1993), either as single agents or in combination (Sher *et al.*, 1994), in an attempt to improve the outcome of subsequent pregnancies. Despite the significant morbidity associated with some of these treatments (Cowchock *et al.*, 1992; Dahlman *et al.*, 1994), there have been no reports in which the efficacy of treatment has been compared to that of placebo. This is of particular importance, as recurrent miscarriage has a high spontaneous resolution rate (Knusden *et al.*, 1991).

The aim of this study was to document the prospective pregnancy outcome of women with a history of recurrent miscarriage (three or more consecutive pregnancy losses), who have (i) persistently positive tests for either LA and/or ACA, (ii) been comprehensively investigated and found to have no other cause to account for their pregnancy losses, and (iii) declined therapeutic intervention in their subsequent pregnancy.

Materials and methods

Protocol for the investigation of recurrent miscarriage

The St Mary's Hospital Recurrent Miscarriage Clinic sees 500 new patients each year. All women attending our clinic are investigated according to our previously described protocol (Clifford *et al.*, 1994). Briefly, both partners had peripheral blood karyotyping performed. The female partner has a pelvic ultrasound scan performed to determine uterine anatomy and ovarian morphology, in particular the presence or absence of polycystic ovaries (PCO). All women had concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH) assayed at mid-follicular phase. Women in whom an ultrasound diagnosis of PCO was made were further investigated by daily early morning urinary LH analysis (Watson *et al.*, 1993). All women were screened for the presence of LA and both the immunoglobulin (Ig)G and IgM classes of ACA. Sample collection and processing for the detection of LA was made in accordance with guidelines issued by the Lupus Anticoagulant Working Party of the British Society for Haematology (Lupus Anticoagulant Working Party on behalf of the BCSH Haemostasis and Thrombosis Taskforce,

1991). The dilute Russell's viper venom time (dRVVT) together with a platelet neutralization procedure (PNP) was used to detect LA. A dRVVT test ratio of ≥1.1 which corrected by at least 10% with the PNP was considered positive for LA. Both the IgG and IgM classes of ACA were assayed using a standardized enzyme-linked immunosorbent assay (Loizou *et al.*, 1985). Results were expressed in either GPL or MPL, arbitrary units derived from the activity of affinity purified sera (Harris, 1990). An IgG ACA value of ≥5 GPL units and an IgM ACA value of ≥3 MPL units were considered positive. These values, which represented the mean +5 SD of 40 normal control sera, were the cut-off values recommended in guidelines issued by the Association of Clinical Pathologists (Khamashta and Hughes, 1993). All women with a positive test for LA and/or ACA were retested at least 8 weeks after the initial positive test. Only women with persistently positive tests were considered to have antiphospholipid syndrome (Harris, 1987).

Subjects

We studied the outcome of pregnancy in 20 women with persistent positive tests for APA and a history of recurrent miscarriage, who declined treatment with either low dose aspirin or low dose aspirin and heparin in their next pregnancy. The median age of these 20 women was 32 years (range 23–41), and they had a median of four previous miscarriages (range 3–11). Of the previous 110 pregnancies in these 20 women 92 (83.6%) had resulted in miscarriage, which had all been first trimester except one which was an intrauterine death at 18 weeks gestation. All 20 women had persistently positive tests for APA (14 LA positive; 3 IgG ACA positive; 2 IgM ACA positive and 1 LA and IgM ACA positive); none fulfilled the criteria for diagnosis of systemic lupus erythematosus (Tan *et al.*, 1982), and none had a history of thrombo-embolic disease. All 20 women had been investigated according to the above protocol and no cause, other than APA, had been found to account for their pregnancy losses. The outcome of pregnancy in the women with APA was compared with that of a control group of 100 consecutive women (median age 33 years; range 23–44) with a history of recurrent miscarriage (median 4; range 3–10) who were also investigated by the same protocol and in whom all investigations were normal. In this control group, 385 of the previous 450 pregnancies (85.6%) had miscarried, all in the first trimester. None of the 120 women in this study received pharmacological treatment, apart from folic acid for prophylaxis against neural tube defects, during their pregnancy. All women were encouraged to attend a dedicated early pregnancy clinic at which supportive care, in the form of counselling, was offered and serial first trimester ultrasound scans were performed. All women were studied over the same 2 calendar years.

Statistical analysis

The outcome of pregnancy and the presence of fetal heart activity prior to a diagnosis of pregnancy failure in the two groups of women were compared using Fisher's exact test.

Results

Of the 20 women with APA, 18 (90%) miscarried, compared with 34 of the 100 women (34%) in whom no underlying cause to account for their previous miscarriages was found ($P < 0.001$). A similar proportion of women in the two groups, 17 out of 20 women with APA (85%) and 94 out of 100 women with normal investigations (94%), attended the EPC.

The gestations of the miscarriages were similar in the two groups. In the women with APA, 17 of the 18 miscarriages (94%) were first trimester losses as were 33 of the 34 miscarriages (97%) in the women with normal investigations. One woman with APA had an intrauterine death diagnosed at 20 weeks gestation and one woman with normal investigations presented with painful contractions and bleeding at 17 weeks gestation. The only woman with APA and a history of second trimester pregnancy loss miscarried in the first trimester.

Amongst women who had a first trimester miscarriage and who attended the early pregnancy clinic, fetal heart activity prior to fetal demise was noted significantly more often in women with APA compared to the control group [12 out of 14 (86%) versus 12 out of 28 (43%); $P = 0.01$]. The gestational age at which the first ultrasound scan was performed was the same in both women with APA and in the control group (median 6 weeks; range 5–8 weeks).

Two of the 20 women (10%) with a diagnosis of antiphospholipid syndrome had a successful pregnancy. Both women were positive for ACA only (one IgG ACA positive, one IgM ACA positive). Both pregnancies were uncomplicated, progressed to term (39 weeks and 40 weeks gestation) and both babies were of normal birthweight (2.9 and 3.4 kg).

Discussion

We have previously reported that 14% of women with a history of recurrent miscarriage are found to have persistently positive tests for APA (Rai *et al.*, 1995). This study documents the prospective pregnancy outcome in 20 such women who received no pharmacological treatment during their pregnancy. The fetal loss rate of 90% emphasizes the need for randomized therapeutic trials to determine the optimum treatment of women with recurrent miscarriage in association with APA. Although only 20 APA positive women were studied, they were representative of a larger cohort of APA positive women with recurrent miscarriage whom we have identified in a prevalence survey of 500 women with recurrent miscarriage (Rai *et al.*, 1995). The majority of women in both the study group and the larger cohort from which they were drawn were (i) positive for LA only and (ii) had suffered recurrent first trimester miscarriages only.

In this study, 17 of the 18 miscarriages (94%) in women with APA occurred during the first trimester. Where ultrasound data were available, fetal heart activity was noted in 86% of cases prior to a diagnosis of pregnancy failure. The most common type of miscarriage in women with APA was therefore the first trimester loss of embryonic, as opposed to anembryonic, pregnancies.

The first successful pregnancies in women with APA were reported to result from treatment with immunosuppressive doses of prednisolone together with low dose aspirin (Lubbe *et al.*, 1983). Since this original report, a variety of treatment regimens have been tried in order to achieve live births in women with recurrent miscarriage and APA. No treatment protocol has been shown to be superior to another. However, two studies have reported a detrimental effect of steroids on pregnancy outcome (Cowchock *et al.*, 1992; Silver *et al.*, 1993). All studies to date have included only small numbers of women and have had different entry criteria. We are currently

carrying out a randomized prospective study comparing the efficacy of treatment with low dose aspirin (75 mg daily) as opposed to treatment with aspirin and unfractionated heparin (5000 units subcutaneously twice daily).

Traditionally, the mechanism of fetal demise in women with APA has been attributed to thrombosis of the utero-placental vasculature and placental infarction (De Wolf *et al.*, 1982; Out *et al.*, 1991). However, these histological findings are not universal and there have been reports of fetal loss in which there was no evidence of placental infarction (Out *et al.*, 1991). The majority of pregnancy losses in women with APA in our study occurred in the first trimester, which suggests that attention should now be focused on the possible effects of APA on embryonic implantation and placentation. Defects in the process of implantation and trophoblast invasion of the maternal decidua and spiral arteries by the blastocyst, are an important cause of early pregnancy failure following both natural conception and in-vitro fertilization. There is accumulating evidence to suggest that APA impair trophoblast function via mechanisms unrelated to thrombosis, and have a direct effect on the placenta. It has been reported that binding of APA to the cytotrophoblast cells, which express phosphotidylserine on their surface, leads to direct cellular injury and inhibition of syncytia formation (Lyden *et al.*, 1992; Rote *et al.*, 1992). In addition, mice which are both actively and passively immunized with monoclonal IgM ACA experience low fecundity and have a high rate of fetal resorption (Sthoeger *et al.*, 1993). This is associated with impairment of implantation and binding of ACA to the trophectoderm.

The fact that two women with ACA had uncomplicated pregnancies implies that there must be some other factor in addition to ACA that determines pregnancy outcome. A major advance has recently been made with the demonstration that ACA requires a plasma protein co-factor, β2 glycoprotein I (β_2GPI), to exert its action (Galli *et al.*, 1990; McNeil *et al.*, 1990). β_2GPI is a plasma protein, molecular weight 50 kDa, which has been demonstrated to have in-vitro anticoagulant properties (Schousboe, 1985). ACA that are the result of an autoimmune process bind β_2GPI and are pathogenic, whereas ACA produced as a result of infection do not bind β_2GPI and are not pathogenic (Hunt, 1992).

The high prospective fetal loss rate in untreated pregnancies of women with APA and a history of recurrent miscarriage suggests that pharmacological intervention in subsequent pregnancies is justified. Prospective randomized studies of sufficient power are needed to determine the optimum treatment for these women. Whilst the association between thrombosis and APA has been demonstrated, the mechanism of fetal loss in women with APA is unknown. As the majority of miscarriages in women with APA occur in the first trimester, attention must be paid to the possible deleterious effects of APA on embryonic implantation and placentation.

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