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Antibodies directed against annexin A2 and obstetric morbidity

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ABSTRACT

Acquired and inherited thrombophilia have both been reported to be associated with an increased risk of obstetric complications in early or later stages of pregnancy. Annexin A2 (ANXA2) is strongly expressed in vascular and placental tissues and plays a crucial role in fibrinolysis. The aim of the present study was to evaluate the prevalence of antibodies directed against ANXA2 in patients with recurrent miscarriage or obstetric complications. Anti-ANXA2 antibodies (aANXA2) were detected by ELISA in the sera from 46 women with obstetric morbidity, mainly recurrent miscarriage. The cut-off value for positivity was defined as 3 standard deviations above the mean optical density (OD) obtained in the sera from 42 female blood donors. The prevalence of aANXA2 in patients and healthy individuals was 15.2% and 2.3%, respectively. A statistically significant difference was observed between the 2 groups in terms of aANXA2 IgG titers ($p = 0.01$). The highest aANXA2 levels were observed in sera from 2 patients with recurrent miscarriage and one patient with preeclampsia. aANXA2 could play a role in thrombotic mechanisms leading to recurrent pregnancy loss and placental vascular disease. Further studies are needed to determine whether ANXA2 is critical for maintenance of placental integrity.

1. Introduction

Acquired and inherited thrombophilia have both been reported to be associated with an increased risk of obstetric complications at early stages of pregnancy, such as recurrent miscarriage, and at late stages of pregnancy, such as intrauterine growth restriction, preeclampsia and intrauterine fetal death (Robertson et al., 2006). The fibrinolytic system is involved in the regulation of trophoblast migration and *in situ* invasiveness (Lala and Chakraborty, 2003). Impaired fibrinolysis during pregnancy has been described in association with obstetric complications including recurrent miscarriage and preeclampsia. Frequent abnormalities of plasma fibrinolytic activators [deficient release of tissue-type plasminogen activator (t-PA) to venous occlusion] and their inhibitors [high plasma concentrations of type 1 plasminogen activator inhibitor (PAI-1)] have been observed in women with early recurrent miscarriage (Gris et al., 1990, 1993). However, in a meta-analysis investigating the association between defects in the fibrinolytic system and recurrent miscarriage, only factor XII deficiency appeared to be significantly associated with recurrent miscarriage (Sotiriadis et al., 2007). The PAI-1 4G/5G polymorphism represents an independent risk factor for recurrent pregnancy loss (Magdoud et al., 2013). Conflicting results have been reported in the literature concerning the role of thrombin activatable fibrinolysis inhibitor (TAFI) in women with recurrent miscarriage. A recent study failed to demonstrate any difference between women with recurrent miscarriage and a control group in terms of blood TAFI antigen levels (Kayatas et al., 2014). Alterations of fibrinolysis have also been observed in preeclampsia (Pinheiro et al., 2013). Histologically, preeclampsia is characterized by infarction and excess fibrin deposition in the placenta. Acosta-Tejeda et al. reported significantly higher plasma TAFIa (active form) levels in 87 women with preeclampsia (Acosta-Tejeda et al., 2011).

Annexins are calcium-dependent, anionic phospholipid-binding proteins (Gerke and Moss, 2002). They have a common structure characterized by a conserved C-terminal core domain, containing four α-helical "annexin" repeats (eight in annexin A6), and a variable N-terminal head domain, which confers individual functional properties to the various annexins (Gerke and Moss,

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2002). Annexins are involved in various cellular processes such as exocytosis, endocytosis, regulation of ion channel activity, and stabilization of membrane domains (Gerke and Moss, 2002). Annexins are abundant in placental membranes (Kaczan-Bourgois et al., 1996). Within the annexin family, annexin A2 (ANXA2) and annexin A5 (ANXA5) possess profibrinolytic (Ling et al., 2004; Ishii et al., 2001) and antithrombotic activities (Ueki et al., 2012), respectively. ANXA5 (otherwise known as placental anticoagulant protein I) was discovered in the placenta, where it acts as an antithrombotic agent and plays an important role in maintenance of placental integrity (Funakoshi et al., 1987). Antibodies directed against ANXA5 have been shown to be associated with obstetric complications in antiphospholipid syndrome (APS) (Galli et al., 2007). ANXA2 is highly expressed in vascular and placental tissues (Hajjar and Krishnan, 1999). Acting as a cell surface co-receptor for plasminogen and t-PA, ANXA2 promotes vascular fibrinolysis (Hajjar et al., 1994). Antibodies directed against ANXA2 have been detected with a significantly higher prevalence in antiphospholipid syndrome than in healthy individuals (Cesarman-Maus et al., 2006; Salle et al., 2008). The nature of immunodominant epitopes has yet to be identified (Salle et al., 2012). Cesarman-Maus et al. found that anti-ANXA2 antibodies (aANXA2) can activate human endothelial cells (Cesarman-Maus et al., 2006) and could therefore play a pathogenic role in thrombosis associated with APS. These data prompted us to evaluate the prevalence of aANXA2 in women with recurrent miscarriage or late obstetric complications of unknown etiology.

2. Materials and methods

2.1. Patients and healthy subjects

Forty six women followed at the Amiens University Hospital Department of Internal Medicine (Amiens, France) were retrospectively included in this case-control study. These women had a diagnosis of placenta-mediated pregnancy complications such as recurrent early pregnancy loss (defined as two or more consecutive abortions of gestational age before 22 weeks of gestational age) (Goodman et al., 2006), intrauterine growth restriction (IUFG), intrauterine fetal death (IUFD) after 22 weeks of gestation (Hoffmann et al., 2012), preeclampsia, eclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, low platelet counts). A control group consisted of forty two healthy female blood donors recruited at the Amiens branch of the French Blood Establishment-North of France. Demographic data were recorded for all women. Some patients had a placental examination and the available histologic data were recorded. The following laboratory data were collected for each woman with pregnancy morbidity: plasminogen; deficiencies of natural anticoagulants: antithrombin, protein C and protein S; activated protein C resistance (APCR); antinuclear antibodies; homocysteinemia; determination of the following gene mutations: factor V [Leiden], factor II (prothrombin) [G20210A], methylene tetrahydrofolate reductase [MTHFR C677T and A1298C] and factor XII [C46T]; IgG and IgM anticardiolipin antibodies (ACL); IgG and IgM anti-β2 glycoprotein I antibodies (aB2GPI), lupus anticoagulant (LA). Serum samples were collected from all women and were stored at −80 °C.

2.2. Detection of anti-ANXA2 antibodies

Antibodies directed against ANXA2 (aANXA2) (IgG and IgM) were detected by an enzyme-linked immunosorbent assay (ELISA), as previously described with minor modifications (Salle et al., 2008). One half of the 96-well plates (Nunc Maxisorp, Nunc A/S, Roskilde, Denmark) were coated overnight at 4 °C with recombinant ANXA2 (1 μg/mL) (ABNOVA, Taiwan). The remaining wells received 100 μL of phosphate buffered saline (PBS). After three washes with PBS/0.1% Tween (PBST), wells were blocked with 3% bovine serum albumin in PBST for 90 min at room temperature. After three washes with PBST, 100 μL of individual patient sera (diluted to 1:50 in blocking buffer) were added to both ANXA2- or PBS-coated wells for 1 h at room temperature. Mouse monoclonal anti-ANXA2 antibody (Invitrogen, Camarillo, California, USA) was used as positive control. Wells were washed three times with PBST and were then incubated with 100 μL of peroxidase-conjugated antibodies (goat anti-mouse IgG, goat anti-human IgG, goat anti-human IgM (Sigma, St. Louis, MO, USA)) (diluted to 1:2000 in PBST) for 1 h at room temperature. After three washes, 100 μL of tetramethylbenzidine solution (Sigma) were added and color development was stopped with 50 μL of H_2SO_4. Absorbance was measured at 405 nm and the results were expressed as optical density (OD), calculated by subtracting the background from wells without ANXA2. The cutoff value for determining positivity of anti-ANXA2 IgG or IgM was defined by 3 standard deviations (SD) above the mean value observed with a serum panel from forty two healthy women.

Table 1
Clinical characteristics of the study population of women.

Characteristics	Number of patients (%)
Recurrent pregnancy loss (≥2)	33 (71)
- early pregnancy loss (≥2)	30 (65)
- late pregnancy loss (>12 weeks of gestation) (≥1)	8 (17)
IUFD (≥22 weeks gestational age)	14 (30)
IUGR	11 (24)
Preeclampsia	5 (10)
Eclampsia	2 (4)
HELLP syndrome	2 (4)

2.3. Detection of anti-ANXA5 antibodies

Anti-ANXA5 IgG and IgM antibodies were detected by a commercial ELISA kit (AESKU.DIAGNOSTICS, Germany).

2.4. Statistical analysis

The proportion of aANXA2 positivity in patients and controls was compared by Fisher's exact test. A Wilcoxon signed-rank test was used to compare aANXA2 IgG and IgM titers in healthy female blood donors and the group of women with obstetric morbidity.

3. Results

3.1. Patient characteristics

A total of eighty eight women were included in the study: forty six women with pregnancy complications and forty two healthy female blood donors. The median age (range) was not statistically different between patients (30.5 years [17–41]) and healthy women (30.5 years [18–61]). Clinical characteristics of patients are summarized in Table 1. Recurrent pregnancy loss represented the most frequent complication in this population of women.

Eight women with recurrent miscarriage presented both early and late pregnancy losses. At least one histologic examination of the placenta was available for seventeen patients and showed ischemic or thrombotic placental disease in nine patients. Laboratory data are shown in Table 2.

The majority of patients who were positive for antiphospholipid antibodies (aPL) became negative. Two patients had persistent aPL and sera were tested for aPL on at least 5 occasions. However, persistent aPL were present in low levels (ACL IgM ≤ 26 U/ml