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Activated protein C resistance, but not antiphospholipid antibodies, is associated with recurrent first-trimester spontaneous abortions

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Background. The aim of the study was to evaluate the association between the most common laboratory markers of thrombophilias and recurrent first-trimester spontaneous abortions.

Materials and methods. During the period from November 2000 to April 2004, a case-control study was carried out at the Department of Obstetrics and Gynecology of Kaunas University of Medicine. Eighty nulliparous women with two or more unexplaned spontaneous abortions ≤13 weeks of gestation and 80 healthy parous women with no history of miscarriages were enrolled into the study. Blood tests for activated protein C resistance (APCR), anticardiolipin antibodies type G (ACLA IgG), anticardiolipin antibodies type A (ACLA IgA) and lupus anticoagulant (LA) were performed in women of both groups. International normalized ratio (INR), activated partial thromboplastin time (APTT), fibrinogen concentration and platelet count were also analysed in all women.

Results. The mean age of patients was 28.1 years in the group of cases and 30.7 years in controls. The mean number of miscarriages was 3.5. APCR was detected in 15% of women with recurrent miscarriage and in 5% of control patients ($p < 0.05$; OR = 3.4). ACLA IgG were found in 22.5% of cases and in 26.3% of controls ($p > 0.05$), ACLA IgA in 1.3% and 5% ($p > 0.05$), respectively. LA was detected in 2.5% women in both groups. APTT was significantly prolonged in women with a positive LA test ($p < 0.05$).

Conclusion. Activated protein C resistance, but not antiphospholipid antibodies, is associated with recurrent first-trimester spontaneous abortions.

Keywords: Activated protein C resistance, antiphospholipid antibodies, recurrent spontaneous abortion, thrombophilia

INTRODUCTION

Recurrent spontaneous abortions affect 1–3% of women of reproductive age (1). The most common causes of miscarriages are parental chromosomal abnormalities, uterine abnormalities, infection and endocrine disorders. Pregnancy loss is reported to be more common in women with inherited and acquired thrombophilias. Antiphospholipid antibody syndrome has been established as an important and treatable cause of recurrent miscarriage (1, 2). Other specific thrombophilias found to be associated with spontaneous abortions are factor V Leiden, methylenetetrahydrofolate reductase, and prothrombin G20210A mutations, deficiencies of protein C, protein S and antithrombin (3, 4). Factor V Leiden mutation results from guanine substitution for adenine at the 1691 position of the gene-encoding factor V. This substitution renders factor V resistant to cleavage by activated protein C (3). In normal clotting, activated protein C inactivates factors Va and VIIIa by cleavage at specific sites. In the presence of factor V mutation, the cleavage of this factor is inhibited, leading to enhanced thrombin generation and increased clot formation. Factor V Leiden mutation is responsible for 50–95% of cases of activated protein C resistance (APCR) and now is the most common known genetic predisposition to thrombosis (3, 5, 6). However, APCR may be present in pregnancy without factor V Leiden mutation (7, 8). The incidence of APCR up to 38% in

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women with recurrent pregnancy loss was reported in several studies (9–11).

The proposed mechanisms of fetal loss in women with thrombophilia include inhibition of the thrombolytic system, thrombosis of placental vessels, placental infarction, and direct cytotoxic effects. The shift in the thromboxane / prostacyclin ratio in favour of the prothrombotic agent thromboxane may lead to vasospasm and platelet aggregation in the trophoblast, causing the development of microthrombi and placental necrosis (8).

The purpose of this study was to evaluate the association between the most common markers of thrombophilia and recurrent early spontaneous abortions in Lithuania, using a case-control design at the Department of Obstetrics and Gynecology of Kaunas University of Medicine.

MATERIALS AND METHODS

During the period from November 2000 to April 2004, a case-control study was carried out at the Department of Obstetrics and Gynecology of Kaunas University of Medicine, a tertiary-care referral centre. Two groups of women were enrolled: 80 women with recurrent early spontaneous abortions as cases and 80 healthy controls from the same ethnic background with no history of spontaneous abortion, who delivered at least one term infant. Inclusion criteria for the cases were nulliparity and more than two spontaneous abortions ≤13 weeks of gestation. Women with anatomical abnormalities of the genital tract, infectious complications, serious trauma at the time of spontaneous abortion and families (or pairs) with chromosomal abnormalities were excluded from the study. Five women were excluded from the study: one due to chromosomal abnormalities (balanced Robertsonian translocation), three due to uterine abnormalities, and one due to the refusal of karyotyping. For each case one control woman with no history of spontaneous abortion and with previous term delivery was recruited, matched with cases for age (±5 years) and body mass index (±3 kg/m^2).

Background data were obtained from cases and controls by personal interview, using a structured questionnaire. Other necessary characteristics were obtained from medical records. Information was collected after admission of a patient by the principal investigator (V.A.). Gestational age was determined by the date of the last menstrual period and by ultrasound measurements.

Blood tests for activated protein C resistance (APCR), anticardiolipin antibodies type G (ACLA IgG), anticardiolipin antibodies type A (ACLA IgA) and lupus anticoagulant (LA) were done in women of both groups. Some coagulation parameters as international normalized ratio (INR), activated partial thromboplastin time (APTT), concentration of fibrinogen and platelet count were also analysed in all women. Blood sample of 4.5 ml for APCR detection was obtained by peripheral vene puncture. Blood was taken and placed in a container with 4.5 ml of trisodium citrate. The citrated blood was centrifuged at 2500 rpm for 30 min. The plasma was collected and frozen at –80^o C using liquid nitrogen for a period no longer than 6 months. Before examining, plasma was thawed in a water basin at a temperature of 37 °C for 15 min. The APCR test was done using the 2nd generation Diagnostica Stago method based on APTT with a STACOMPACT automated coagulometer (1994). In order to ensure the normal starting concentration of other coagulation factors, the test plasma was mixed with a factor V deficient plasma. Clotting was initiated with *Croalus viridis helleri* poison which activates factor X. Plasma with the clotting time of 120 s or more was evaluated as APCR-negative, whereas plasma whith clotting time less than 120 sec was evaluated as APCR-positive.

Antiphospholipid antibodies were analysed with a STACOMPACT automatic coagulograph (1994).

The normal range of APTT with STAGO LUPUS ANICOAGULANT SENSITIVE APTT reagent was 37.4 ± 6 s and the pathological range was more than 44 s. A mixed test was performed in women with APTT more than 38 s and APTT-LA time more than 44 s. If the APTT value did not return to the normal range, the test was evaluated as positive for LA.

ACLA type IgM and IgA were detected by an enzyme-linked immunosorbent assay (ELISA). The presence of antibodies was detected at IgG levels more than 5 GPL or IgM more than 3 GPL. The concentration of ACLA was detected with a specific reagent "Bindazym", using an IMMULITE analyzer (2000). The test was evaluated as negative when the concentration was less than 14 GPL/ml, as positive at more than 14 GPL/ml, and strongly positive at more than 80 GPL/ml. Women with positive ACLA and LA tests were re-examined after 8 weeks. Therefore, the diagnosis was made when the test was positive on two occasions 8 weeks apart.

Statistical analyses were carried out by the SPSS software (Statistical Package for Social Sciences) 8.0 for Windows (Chicago, Illinois, USA). Relationships between the variables were measured by calculating odds ratios with 95% confidence intervals and correlation coefficient. Student's t test and McNemar's corrected chi-square test were used when appropriate. A value of $p < 0.05$ was considered significant.

The independent Ethics Committee of Kaunas University of Medicine approved the study. Signed informed consent to participate in the study was obtained from all women.

RESULTS

The mean age of the patients was 28.1 years (range, 18–42) in the group of cases and 30.7 years (range, 22–45) in controls, indicating that cases were satisfactorily

matched with controls. The mean number of miscarriages was 3.5 (range, 2–9). The median number of deliveries in the group of controls was 1.4 (range, 1–3). The mean body mass index was 21.5 kg/m^2 in cases and 20.1 kg/m^2 in controls. All women in both groups were Caucasians. Main background data on the women are presented in Table 1.

The prevalence of laboratory markers of thrombophilia is presented in Table 2. The APCR test was positive in 15% of women with recurrent miscarriage and in 5% of controls ($p < 0.05$, OR = 3.4). ACLA IgG and ACLA IgA were more common in women of the control group: ACLA IgG were found in 22.5% of cases and in 26.3% of controls, ACLA IgA in 1.3% and 5%, respectively; the differences were not significant. LA was found in 2.5% of women in both groups.

Some blood coagulation parameters in cases and controls are presented in Table 3. APTT, INR and fibrinogen level in women of both groups with positive tests for ACLA IgG, LA and APCR are presented in Table 4. APTT was significantly prolonged in women with a positive LA test ($p < 0.05$). APTT was similar in women with positive and negative tests for ACLA IgG and APCR. INR and fibrinogen level were detected in similar ranges in women who were both positive and negative for ACLA IgG, LA and APCR.

The correlation between the quantitative value of APCR and the gestational week at which spontaneous abortion occurred is presented in Fig. 1. The quantitative value of APCR showed a positive correlation with gestatonal week ($r = 0.223$, $p > 0.05$). The correlation between the quantitative value of ACLA IgG and the

Table 1. **Background data on women with recurrent miscarriages and controls**

Characteristics	Cases (n = 80)	Controls (n = 80)	p
Mean age (years)	28.1	30.7	NS
Mean number of miscarriages	3.5	–	–
Median number of deliveries	–	1.4	–
Body mass index (kg/m^2)	21.5	20.1	NS
Nationality (%)			
Lithuanian	86.3	98.8	NS
Russian	11.3	1.2	–
Ukrainian	1.2	–	–
Tatar	1.2	–	–

NS – statistically not significant.

Table 2. **Laboratory markers of thrombophilia in women with recurrent spontaneous abortions and controls**

Criteria	Cases (n = 80)		Controls (n = 80)		OR	95% CI
	No	%	No	%		
APCR	12*	15.0	4	5.0	3.4	1.94–13.01
ACLA IgG	18	22.5	21	26.3	0.8	0.37–1.79
ACLA IgA	1	1.3	4	5.0	0.2	0.01–2.36
LA	2	2.5	2	2.5	1	0.10–10.24

$p < 0.05$.

Table 3. **APTT, INR and fibrinogen in women with recurrent abortions and controls**

Criteria	Cases (n = 80)	Controls (n = 80)	p
	x ± SD	x ± SD	
APTT (s)	35.3 ± 3.8	36.0 ± 3.6	NS
INR	1.0 ± 0.1	1.0 ± 0.09	NS
Fibrinogen (g/l)	3.2 ± 0.7	3.6 ± 0.6	NS
Platelet count (×10^9/l)	194.8 ± 24.2	211.7 ± 34.5	NS

Table 4. **APTT, INR and fibrinogen in women positive and negative for ACLA IgG, LA and APCR**

Parameter	ACLA IgG			LA			APCR		
	Positive (n = 39) x ± SD	Negative (n = 121) x ± SD	p	Positive (n = 4) x ± SD	Negative (n = 156) x ± SD	p	Positive (n = 16) x ± SD	Negative (n = 144) x ± SD	p
APTT (sec.)	36.0 ± 3.6	35.5 ± 3.5	NS	41.1 ± 4.2	34.2 ± 3.2	< 0.05	36.0 ± 3.5	34.8 ± 3.1	NS
INR	1.0 ± 0.1	1.0 ± 0.01	NS	1.0 ± 0.1	1.0 ± 0.1	NS	1.0 ± 0.1	1.0 ± 0.1	NS
Fibrinogen(g/l)	3.2 ± 0.5	3.4 ± 0.7	NS	3.1 ± 0.6	3.3 ± 0.5	NS	3.3 ± 0.7	3.5 ± 0.5	NS

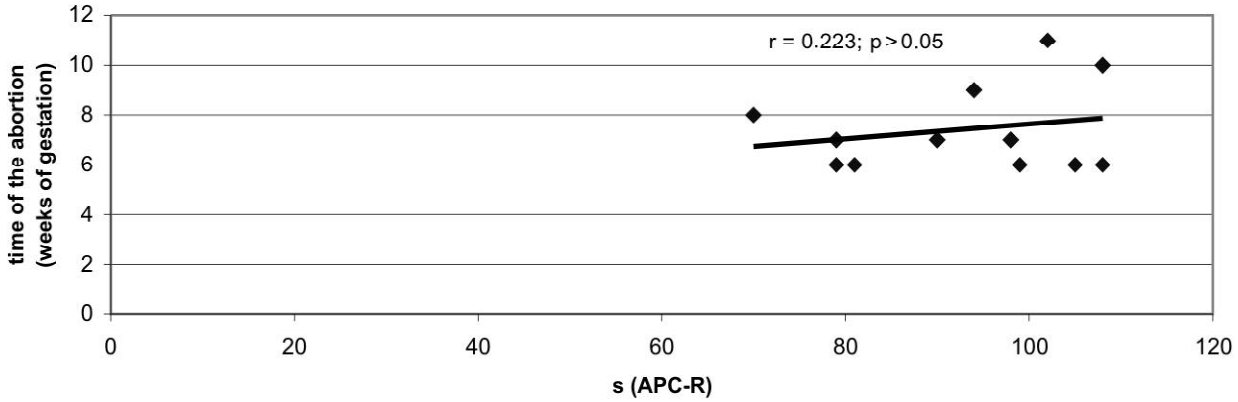

Fig. 1. Correlation between quantitative value of APCR and gestational week

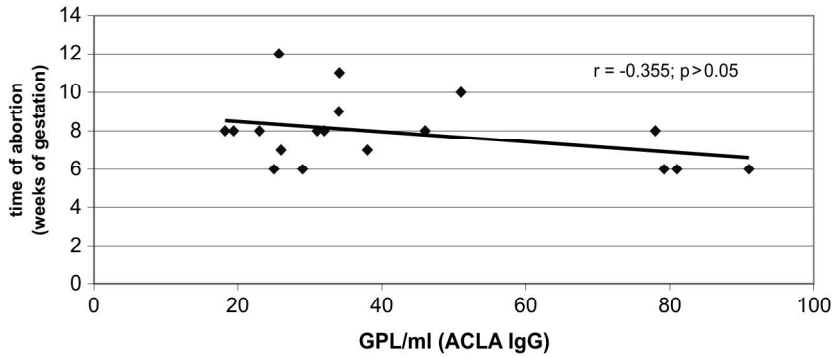

Fig. 2. Correlation between the quantitative value of ACLA IgG and gestational week

gestational week at which spontaneous abortion occurred is presented in Fig. 2. The quantitative value of ACLA IgG had a negative correlation with gestatonal week ($r = -0.355$, $p > 0.05$).

DISCUSSION

Recurrent miscarriage is a significant clinical problem. Recently, several inherited and acquired thrombophilic disorders have been implicated as a possible cause. Sarig et al. reported at least one thrombophilic defect in 66% of women with recurrent miscarriage versus 28% in control patients (6). APCR is the most common type of hereditary thrombophilia with an established role in the pathogenesis of recurrent spontaneous abortion. Acquired APCR (without factor V mutation) was found in 9–38% of women with unexplained recurrent pregnancy loss versus 0–3% of controls (9–11). A large number of case-control studies found up to 30% of factor V Leiden mutations in women with recurrent pregnancy loss and in 1–10% of control women, with odds ratios ranging from 2 to 5 (12–14). Rai et al. investigated acquired APCR among 1000 women with recurrent miscarriages, which is the largest published patient group (5). Acquired APCR was found among 8.8% of women with a first-trimester abortion and in 3.3% of controls. Congenital APCR resulting from the factor V Leiden mutation was detected in similar ranges

in both groups in this study. A meta-analysis, which included 31 observational studies, reported a 3-fold increased risk of early recurrent fetal loss in women with APCR (10). Wramsby et al. reported 27.8% of factor V Leiden mutation among women with recurrent abortions (12). Conversely, several other investigators did not find any association between factor V Leiden and fetal loss (15–17). Kutteh et al. did not find factor V Leiden mutation to be more frequent in women suffering from first trimester recurrent pregnancy loss compared with controls (16). APCR was detected in 15% of women with recurrent miscarriage and in 5% of controls in our study; the difference was statistically significant.

There are controversial data on whether APCR is more associated with first-trimester or with second-trimester miscarriage. The results of meta-analysis have suggested that factor V Leiden carriers have a higher risk of late pregnancy loss than first trimester abortion (10). Second- and third-trimester losses were significantly more frequent in women with thrombophilia in the study made by Sarig et al. (6). Tal et al. reported 35% of all fetal losses in women with APCR as "preclinical" (before ultrasound confirmation of fetal heart activity) versus 12% of those in unaffected women (18). Younis et al. prospectively evaluated 37 women with recurrent first-trimester miscarriage and 41 women with second trimester pregnancy loss (19). The incidence of APCR was similar in the first and the second-trimester miscarriage groups and the difference was not significant. Foka et al. also found the APCR to be more prevalent in the trimester than the first trimester abortion (20). The available evidences suggest an increased risk of fetal loss in women with APCR throughout pregnancy, although the risk may be higher in the second trimester. In our study, only women with first-trimester abortions were investigated.

The prevalence of APCR is different among populations and some races. It is low in Asian and African populations but considerably higher in populations of Caucasian origin with a 2–9% incidence. An extremely high incidence (up to 15%) was reported in Scandinavia (21). All women included into our study as cases and controls were Caucasians.

Antiphospholipid antibodies can be found in completely healthy women (22, 23). Ober et al. reported 28% of positive tests for at least one of the antiphospholipid antibodies in women without history of recurrent abortions (22). Antiphospholipid antibodies were reported to be associated with 5–40% of recurrent pregnancy loss (2–4). All types of antiphospholipid antibodies (ACLA IgG, ACLA IgA and LA) were not associated with recurrent spontaneous abortions in our cases. The prevalence of ACLA IgG and ACLA IgA among control patients was even higher than in women with recurrent spontaneus abortions, but the difference was not significant. The prevalence of LA was the same (2.5%) in women with recurrent pregnancy loss and controls.

CONCLUSION

Activated protein C resistance, but not antiphospholipid antibodies, is associated with recurrent first trimester spontaneous abortions.

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ATSPARUMAS AKTYVINTAJAM C BALTYMUI, BET NE ANTIFOSFOLIPIDINIAI ANTIKŪNAI, YRA KARTOTINIŲ ANKSTYVŲJŲ SAVAIMINIŲ PERSILEIDIMŲ PRIEŽASTIS

Santrauka

Darbo tikslas – ištirti dažniausius laboratorinius trombofilijų žymenis moterims, patyrusioms kartotinius ankstyvuosius savaiminius persileidimus, ir palyginti duomenis su kontrolinės grupės tyrimų rezultatais.

Darbo metodika. Nuo 2000 m. lapkričio iki 2004 m. balandžio Kauno medicinos universiteto Akušerijos ir ginekologijos klinikoje atliktas atvejo ir kontrolės tyrimas. Ištirta 80 pacienčių, patyrusių du ir daugiau savaiminių persileidimų iki 13 nėštumo savaitės, ir 80 sveikus išnešiotus naujagimius pagimdžiusių moterų. Visoms moterims ištirtas atsparumas aktyvintajam C baltymui (AACB), antifosfolipidiniai antikūnai – *Lupus* antikoagulantas (LA), G grupės antikardiolipiniai antikūnai bei A grupės antikardiolipidiniai antikūnai. Taip pat ištirtas tarptautinis normalizuotas santykis (TNS), aktyvintojo dalinio tromboplastino laikas (ADTL), fibrinogeno koncentracija ir nustatytas trombocitų skaičius periferiniame kraujyje.

Darbo rezultatai. Vidutinis kartotinius persileidimus patyrusių moterų amžius buvo 28,1 metų, kontrolinės grupės moterų – 30,7 metų. Tiriamosios grupės moterys buvo patyrusios vidutiniškai 3,5 savaiminių persileidimų. AACB nustatytas 15% moterų, patyrusių kartotinius ankstyvuosius savaiminius persileidimus, ir 5% kontrolinės grupės moterų ($p < 0,05$; ŠS = 3,4). ACLA IgG nustatyti 22,5% persileidusių moterų ir 26,3% kontrolinės grupės moterų ($p > 0,05$), ACLA IgA – atitinkamai 1,3% ir 5% moterų ($p > 0,05$). LA nustatytas vienodai dažnai, t. y. 2,5% abiejų grupių moterų. Moterims, turinčioms LA, kur kas dažniau nustatytas pailgėjęs ADTL ($p < 0,05$).

Išvada. Atsparumas aktyvintajam C baltymui, bet ne antifosfolipidiniai antikūnai, gali būti kartotinių ankstyvųjų savaiminių persileidimų priežastis.

Raktažodžiai: atsparumas aktyvintajam C baltymui, antifosfolipidiniai antikūnai, kartotinis savaiminis persileidimas, trombofilija