

Activated protein C resistance and pregnancy – Department's of Haematology, Coltea Clinical Hospital experience

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ABSTRACT

Activated Protein C resistance (APCR) is the most frequency trombophilia that induces thrombotic complications of pregnancy that itself is a hypercoagulable state. Abnormal placental vasculature is the most important mechanism that causes various complications. The screening diagnosis of APCR is influenced by physiological changes of hemostasis in pregnancy and may be difficult. We propose to evaluate the risk for thrombosis complications associated with the factor V Leiden mutation in pregnancy, using a lot of women with complications of pregnancy selected from our casuistic. We concluded that factor V Leiden mutation and resistance to activated protein C are important risk factors for pregnancy complications. The screening for resistance to activated protein C with FV deficiency plasma is recommended in all pregnant women with vascular placental complications or history of pregnancy complications.

Keywords: activated protein C resistance, factor V Leiden, pregnancy complications

INTRODUCTION

Activated Protein C resistance and factor V Leiden

Activated Protein C resistance (APCR) was first described in 1993 by Dahlback (1) who observed that certain patients with recurrent thrombosis did not prolong the activated partial thromboplastin time (APTT),

when activated protein C (APC) was added to their plasma. This phenomenon was explained by autoantibody against Protein C, antiphospholipid antibodies inhibiting APC function or Protein S deficiency. In 1994 Bertina identified a genetic abnormality of factor V, named Leiden anomaly that is inherited in an autosomal dominant manner and induces APC resistance. In factor V Leiden, the arginine at position 506 is substituted with glutamine and

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this conduce towards an activated Factor V molecule resistant to cleavage by APC. The procoagulant activity of activated Factor V is normal. Further, another genetic mutations of FV which lead to APCR and contribute to an increased risk of thrombosis (R2 allele, FV Cambridge R306T mutation or factor V Hong Kong R306G mutation) (2,3) have been identified. In spite of all, the factor V Leiden variant incidence is the highest (about 5% – 12% in general population) and it is considered responsible for up 95% of cases of APC resistance. The risk of thrombosis is a 7.9 fold increased for heterozygous and a 91 fold increased for homozygous with Factor V Leiden mutation that is considered the most frequently thrombophilia being found in 20 to 60% of patients with recurrent thrombosis.

Acquired resistance to activated protein C

There are many conditions that lead up to APC resistant without any mutations: oral contraceptive use, pregnancy, inflammatory diseases, acute thrombotic events and cancers (4). Lupus anticoagulants are also associated with a reduced sensitivity to APC (5). Demographic factors, female gender, elderly, obesity, high cholesterol, high triglycerides and hypertension have a positive correlation with APC resistance (4). Mechanisms of acquired APC resistance described are: increased plasma levels of factor VIII in acute-phase reactions, in patients with acute thrombotic events or in cancer patients (6), high factor VII levels in oral contraceptive users or lupus anticoagulants inhibition on the action of APC on factor V (7). The pregnancy is associated with increased resistance to activated protein C dependent on elevated coagulations factor VIII and V levels but this is not real risk factor for venous thrombosis (8).

Haemostatic changes in pregnancy

Normal pregnancy and puerperium are characterized by a marked increased in the procoagulant activity in maternal blood (9). Virchow's triad in normal pregnancy is characterized by venous stasis, venous hypotonia or vascular damage and hypercoagulability. The most blood coagulation factors and fibrinogen increase during pregnancy: FVII and FX are mild increasing, Fibrinogen and FVIII are 2 fold increasing, von Willebrand Factor increases 3-fold and remains elevated some period post partum

and FV gradual rises (10). FXII, X and IX increase progressively in contrast with FXI that is the only blood coagulation factor that decrease. Tissue factor (TF) no change and it has a VTE protecting role in pregnancy. Another hypercoagulability causes in pregnancy are the natural coagulation inhibitors changes: total and free protein S decrease about 30% and may remain decreased for at least up to 2 months postpartum, protein C remain constant or increase but Heparin cofactor II and Thrombomodulin increase in pregnancy. Level of ATIII remains stable during pregnancy. Fibrinolytic capacity is diminished during pregnancy, mainly because of markedly increased levels of plasminogen activator inhibitor-1 (PAI-1) from endothelial cells and plasminogen activator inhibitor-2 (PAI-2) from the placenta (11). The changes in the haemostatic system progress with pregnancy evolution and are maximal around term; its help in maintaining placental function during pregnancy, minimizing intrapartum blood loss and preparing the haemostatic challenge of delivery. Haemostatic system returns to non-pregnant state in 4 – 6 weeks post-delivery. The incidence of VTE in pregnancy is 1/1000 deliveries (6 fold higher than in general female population of child-bearing age). An important anticoagulant mechanism changed is acquired APC resistance that was reported in up to 50% of normal pregnancies. The cause of this change is the increase level of FVIII and FV, decrease level of PS or APC inhibitors (12).

Complications induced by APCR in pregnancy

Normal pregnancy is characterized by acquired activated Protein C resistance, but this hypercoagulable state doesn't induce thrombotic complications and doesn't need antithrombotic prophylaxis. Instead, the inherited activated Protein C resistance induced by FV Leiden has often complicated pregnancy. The mutation induce a three to four fold higher risk of an adverse pregnancy outcome (13) and has a stronger association with severe and early-onset preeclampsia (14, 15). Also, recurrent miscarriages, defined as three early consecutive losses or two late pregnancy losses after 12-weeks gestational age (16) has been shown to be associated with APCR. The data on the risk of intrauterine fetal growth restriction (IUGR) are more limited and conflicting (17, 18).

Placental abruption or maternal venous thrombosis during pregnancy or postpartum is another described complications (19, 20). The causes of these are unknown but all of them may be associated with abnormal placental vasculature and disturbances of hemostasis leading to inadequate maternal-fetal circulation (21). Another possible mechanism is cell death and inhibition of trophoblast cells growth induced by activated coagulation factors (22). Clinical factors that increase these complications risk in pregnancy are: maternal age, maternal weight, high parity, major current illness and operative delivery.

APCR laboratory diagnosis

The classic method described by Dahlback consists in ratio of activated partial prothrombin time (APTT) with Protein C activator and simple APTT (APTT with APC/APTT). This test is influenced by pregnancy due factors level increased and protein S deficiency. The FV deficient plasma method (APCR V) covers the influences of pregnancy or other conditions about test and is indicated in these situations. The certainty diagnosis of Leiden anomaly consists in genetic tests for FV mutations through Polymerase Chain Reaction – PCR – for FV Leiden – G1691A – Arg506Gln.

The management of APCR during pregnancy

The primary thromboprophylaxis in asymptomatic women with known FV Leiden mutation, who have never experienced VTE, consist in clinical surveillance or prophylactic therapy during the last weeks of pregnancy and 2–6 weeks in the puerperium with heparin or low molecular weight heparin (LMWH) (21). Secondary prophylaxis of recurrences in women who have previously developed thrombosis consists in active prophylactic therapy with heparin or LMWH and clinical surveillance (23), especially for women who exhibit additional risk factors such as hyperemesis, obesity, immobilization or surgery. The treatment of acute thrombotic episodes during pregnancy in women with or without Leiden anomaly is with full dose intravenous heparin for 5–10 days, adjusted to prolong the activated partial thromboplastin time (aPTT) into the therapeutic range, followed by maintenance subcutaneous heparin given twice daily (24). Antithrombotic

prophylaxis with low dose Aspirin was described with similar effect (25).

The purpose of this study was to investigate the relationship between various pregnancy complications and APCR induced by FV mutations, in a lot of women with obstetrical complications selected from our Department casuistic. □

MATERIAL AND METHODS

This was a retrospective study in a lot of 623 women with pregnancy complications selected from Coltea Hematology Department casuistic in last 3 years (October 2005 – September 2008). The included criteria were: one or more miscarriages previous 12 weeks pregnancy, second or third trimester fetal loss, pre-eclampsia, VTE during pregnancy or in puerperium, intrauterine fetal growth restriction and increased of uterine arterial flux. Exclusion criteria included: induced abortions, infections, systemic disease or uterine structural abnormalities, fetal malformations, uterine col dehiscence, hypertension previous pregnancy, another inherited thrombophilia (protein C, S and ATIII deficiency) and antiphospholipidic syndrome. All data about personal and family history of thrombosis or pregnancy complications and other risk factors for thrombosis including smoking and oral contraceptive usage were collected from medical papers of patients and were included in an Excel database. Other demographic data, age, number and outcome of pregnancies and the treatment were included in database. The results of screening coagulation tests (prothrombin time – PT -, activated thromboplastin time – APTT – and fibrinogen) protein C, protein S, ATIII, Lupus anticoagulant and APCRV test were extracted from database of our Department Laboratory. All these coagulometric tests were performed on ACL 9000 automated coagulometer using Instrumentation Laboratory reagents. Blood was obtained from fasting patients in sodium citrate vacuum blood collection tubes. Plasma was obtained by centrifugation at 1500 g for 15 minutes; it was then frozen and stored at – 20 °C. All measurements were subsequently performed within 2 weeks of collection. Resistance to activated protein C was measured as the activated protein C sensitivity ratio using FV deficient plasma (APCRV) in an ACL 9000 coagulometer. □

RESULTS AND DISCUSSIONS

Clinical characteristics of the patients from study lot are shown in Table 1. The median age of patients was 31 years with range 19 to 43 years; number of pregnant women in testing moment was 458. The antiphospholipid syndrome, deficiency of PC, PS and ATIII were excluded for all patients from study lot. In 72 cases the APCRV test was positive and suspicious of FV Leiden was risen. Unfortunately the genetic test for this suspicious was performed just in 29 cases and confirmed the diagnosis; all the other cases with APCRV test positive was consider carriers of FV Leiden or another FV mutation. The prevalence of carriers of the factor V mutation in this study lot was 11,55%.

Characteristics	Study lot (n = 623)
Age (median/range)	31 years/19 to 43 years
Pregnant women in testing moment	458 cases (63,5%)
Smoking	219 cases (35,2%)
CO users	293 cases (47%)
Another thrombotic risk factor (obesity, immobilization, surgery)	186% cases (29,9%)
Personal history of VTE without pregnancy	51 cases (8,9%)
Family history of venous thromboembolism	68 cases (10,9%)

TABLE 1. The anamnesis characteristics of women from study lot

Pregnancy complication	Carriers of the Factor V Mutation (n = 72) Number of cases/ Percent	Non – carriers of the Factor V Mutation (n = 551) Number of cases/ Percent
Recurrent miscarriages (>3)	14 cases (19,4%)	85 cases (15,4%)
One pregnancy loss < 12 weeks of pregnancy	34 cases (47,2%)	254 cases (46,1%)
Late pregnancy losses > 12 weeks of pregnancy	11 cases (15,3%)	58 cases (10,5%)
Preeclampsia	4 cases (5,6%)	17 cases (3,1%)
Maternal venous thrombosis during pregnancy or postpartum	9 cases (12,6%)	21 cases (3,8%)
Intrauterine fetal growth restriction (IUGR)	43 cases (59,7%)	176 cases (31,9%)
Abnormal placental vasculature (high resistance index in uterine arteries or high maturity grade of placenta)	29 cases (40,3%)	198 cases (35,9%)

TABLE 2. The incidence rates for various obstetric complications for carriers and non-carriers of the factor V mutation

	Carriers of F V mutation (Number and percent of cases with miscarriages)	Non-Carriers of F V mutation (Number and percent of cases with miscarriages)
< 25 years age	9 cases (18, 7%)	87 cases (25,7%)
25 – 30 years age	7 cases (14,6%)	75 cases (22,1%)
30 – 35 years age	13 cases (27,1%)	93 cases (27,4%)
> 35 years age	19 cases (39,6%)	84 cases (24,8%)

TABLE 3. The incidence of miscarriages depend on 5-years intervals in carriers and non – carriers FV mutation women

Pregnancy complications criteria used for analysis in study lots was: recurrent miscarriages (>3), one pregnancy loss < 12 weeks of pregnancy, late pregnancy losses > 12 weeks of pregnancy, preeclampsia, maternal venous thrombosis during pregnancy or postpartum, intrauterine fetal growth restriction (IUGR) and abnormal placental vasculature (high resistance index in uterine arteries or high maturity grade of placenta). The most frequent complication in our study lot was “one early pregnancy loss” (46,23% of cases) followed by abnormal placental vasculature (36,44% of cases).

The women of study lot was divided in two subgroups depend on APCRV test results: subgroup A = 72 cases with positive APCRV test and subgroup B = 551 cases with negative APCRV test.

A family history of thrombosis (recurrent or single episode peripheral venous thrombosis, stroke or pulmonary thromboembolism) was found in 6 women (8,33%) with the positive APCRV test and in 28 women with negative test (5,08%). No difference in thrombosis risk factors (smoking, oral contraceptives, and obesity) was found between those two subgroups.

We have tried to associate the incidence of the different obstetric complications in both

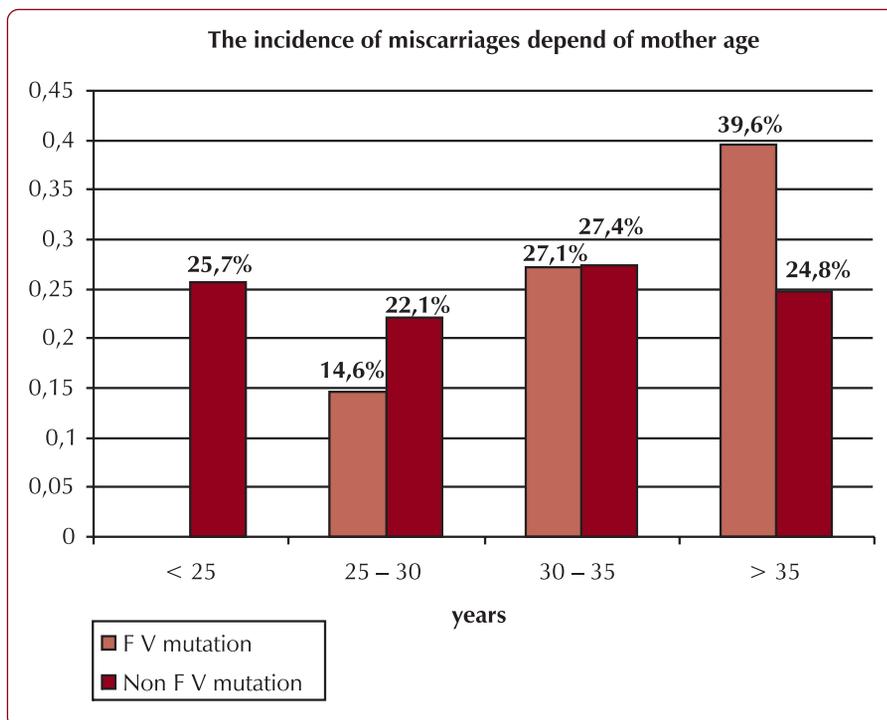


FIGURE 1. The correlation between mother age and obstetrical complications in carriers and non-carriers of FV mutation groups.

subgroups (carriers and noncarriers of the factor V mutation) (Table 3). The most frequent complication for carriers FV Leiden subgroup was “intrauterine fetal growth restriction (IUGR)” and “one early pregnancy loss” for non-carriers FV mutation subgroup. For all obstetrical complications, the incidence was obvious bigger in FV mutation carriers subgroup, except the “one early pregnancy loss” criteria when the incidence was similar in both subgroups. This was in accordance with some previous studies (8,26,27) which report an association of FV Leiden mutation with late than early pregnancy complications.

This study also highlights the low incidence for venous thromboembolism caused by pregnancy in female carriers of the factor V mutation (TABLE 2).

We correlated the incidence of pregnancy complications depend on maternal age. In both subgroups we calculated the incidence/five year's intervals for recurrent miscarriage (TABLE 3).

The analysis of data indicated that two third of FV mutation carriers had recurrent miscar-

riage after 30 years of age, whereas only one third of carriers had this complication before 30 years age. This difference wasn't found in non-carriers of FV mutation subgroup. Our data indicate that women carriers of factor V Leiden mutation develop obstetrical complication more frequent after 30 year of age (FIGURE 1). □

Conclusion

The factor V Leiden mutation and phenotypic response to activated protein C are important risk factors for pregnancy complications. The incidence of Leiden anomaly in our lot is similar with literature, although this is not a really representative lot. The most frequent complications were early pregnancy loss and abnormal placental vasculature. FV Leiden induces late pregnancy losses more frequent than early pregnancy losses and the incidence of miscarriages is increased with maternal age. Our suggestion is that all women with previous complicated pregnancy, with personal and/or family history of thrombosis have indication for screening test of this thrombophilia. □

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