

IS FACTOR V LEIDEN A RISK FACTOR FOR FETAL LOSS?

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Summary: A successful pregnancy is dependent on the development of adequate placental circulation. The abnormalities of placental vasculature may result in a number of gestational pathologies, including fetal loss. The aim of our study was to determine whether women with f V Leiden are at an increased risk of pregnancy loss. For this purpose we assessed three groups of women. In a prospective group we examined 30 females with spontaneous abortions for f V Leiden. In a retrospective group we assessed the frequency of abortions in 80 women (172 pregnancies) with f V Leiden (72 heterozygous, 8 homozygous) from 57 unrelated families. In a control group we evaluated the frequency of abortions in 45 women without f V Leiden. Factor V Leiden was found in 3% of women in the 1st group. Fetal loss occurred in 10% of women in the 2nd group and in 9% in the 3rd group. Factor V Leiden was not found to be a risk factor for fetal loss in our study group.

Key words: Placental infarction; Hypercoagulable state; APC resistance; F V Leiden; Venous thromboembolism; Fetal loss

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Introduction

Deep venous thrombosis is a serious but rare vascular complication during pregnancy and puerperium and pulmonary embolism is one of the most important causes of maternal mortality (10,15).

The physiological changes in haemostasis increase the risk of thromboembolism in pregnancy (16). The risk is even higher in women with congenital thrombophilia (6,13). In 1993 Dahlbäck et al. (9) identified a new mechanism causing inherited thrombophilia, characterized by a poor anticoagulant response to activated protein C (APC resistance-APC-R). The molecular defect underlying this phenomenon was identified as an amino acid substitution at the cleavage site of factor V gene (1691 G to A) (2). Point mutation in the f V gene is responsible for APC-R in more than 90% of cases (14). APC-R without f V Leiden is called „acquired“ APC-R and is usually found in the association with pregnancy (19), use of oral contraceptives (20) and in cancers (12). This imbalance of the haemostatic equilibrium increase the life long risk of thrombosis 5-10 fold in heterozygotes and 50-100 fold in homozygotes (7). In Caucasians, APC-R is the most common of the known inherited risk factors for venous thromboembolism (VTE) (8,25). Women with APC-R and f V Leiden have 8 fold increased risk of VTE in pregnancy (18).

A successful outcome of pregnancy requires an efficient uteroplacental vascular system. Congenital thrombophilia may lead to hypercoagulable state. This state can cause placental infarctions. Placental infarctions can result in the following complications in pregnancy: abortion, intrauterine fetal growth retardation, premature delivery, preeclampsia and intrauterine death (4). Placental thrombosis was previously described not only in women with lupus anticoagulant, but also in women with Antithrombin III (AT III), protein C and protein S deficiencies (23).

The reported early loss rate among recognized pregnancies is between 12%-15% (24). Approximately 5% of women have the experience of two or more consecutive abortions (3). The recurrent fetal loss defined by at least three successive abortions affects 1% to 2% of women at the reproductive age (3).

The aim of our study was to assess the association of APC-R and f V Leiden with spontaneous abortion. This relationship was not known at the time of the beginning of our study. In case that a positive relationship will be found, we want to consider the usefulness of prophylactic anticoagulation treatment in the next pregnancy. As a miscarriage was considered fetal loss before 28th week of pregnancy, as a stillbirth a termination of pregnancy after 28th week. As mentioned above, the recurrent fetal loss was defined by at least three successive abortions.

Materials and methods

In 1996 we examined 500 blood donors (309 males, 191 females) to get prevalence of APC-R in the East Bohemian region. This prevalence has been found to be 1,6%. We assessed the association of APC-R and f V Leiden with spontaneous fetal loss in three cohorts of women.

1. A prospective group

Thirty women admitted to our hospital for spontaneous or recurrent fetal loss were tested for APC-R and f V Leiden. All females completed the questionnaire about family and personal history of VTE. Characteristics of this group are shown in table 1. All women were also tested for protein C, protein S, AT III levels, for the presence of lupus anticoagulant (LA) and anticardiolipin antibody (ACA).

2.A retrospective group

In this group we assessed the course of 172 pregnancies in 80 women with

APC-R and f V Leiden (72 heterozygous, 8 homozygous) from 57 unrelated families. Diagnosis of APC-R and f V Leiden was made either in the laboratory work-up of women with personal history of thrombosis event or in the laboratory work-up of family members of individuals with personal history of thrombosis and with APC-R and f V Leiden. Characteristics of this group are shown in table 2. All women with history of fetal loss were also tested for ACA, LA, protein C, protein S and AT III.

3.A control group

In this group we evaluated the course of 104 pregnancies in 45 women without APC-R and f V Leiden from 42 unrelated families. These women are relatives of individuals with history of thrombotic event and with diagnosis of APC-R and f V Leiden. Characteristics of this group are shown in table 3. All women with the history of pregnancy loss were tested for ACA, LA, protein C, protein S and AT III as well.

Methods: Blood samples were collected by venipuncture into plastic tubes containing either 1/10 volume of 3,8% sodium citrate for coagulation assays or 1/10 volume of 0,5 M sodium EDTA for DNA extraction. After centrifugation (15 min. at 2500 G) for prothrombin time (PT), activated partial thromboplastin time (APTT) and AT III assays or after double centrifugation (+10 min at 1500 G) for protein C, protein S, APC-R and LA assays, citrated plasma was either analyzed immediately (PT, APTT, LA) or stored at -70°C until analyzed (AT III, protein C, protein S, APC-R). APC-R was determined by COATEST APC RESISTANCE kit (Chromogenix). Low response for APC-R was defined as $SR < 2,05$ ($SR = \text{sensitivity ratio-clot time APTT} + \text{APC to APTT without APC}$).

Protein C and protein S were determined by coagulation assays using STACLOT PROTEIN C and STACLOT PROTEIN S kits. AT III was determined by chromogenix

assays using STA-STACHROM AT III kit. All kits are from STAGO Diagnostics. To detect LA, the following assays were performed: PT, APTT (PTT Automate, Stago D.), APTT with high sensitivity to LA (PTT-LA, STAGO D.), TTIT (Tissue Thromboplastin Inhibition Time), dRVVT (diluted Russell's Viper Venom Time). A solidphase immunoassays technique was used to quantify anticardiolipin levels. IgG level > 10 U/ml and IgM level > 7 U/ml were considered as positive results. PCR method was used for f V Leiden determination.

Statistical analysis: Data were evaluated by software program NCSS 6.0.1. using Fisher's test for categorial variables.

Results

1. The prospective group

In the group of 30 women admitted to the hospital we obtained the results which are shown in table 4. Stillbirths did not occur in any of these women. LA, protein C, protein S, AT III deficiencies were not detected either. Frequency of APC-R has been found to be 7% (1%-22%), frequency of f V Leiden 3% (0%-17%).

2. The retrospective group

By the assessment of 172 pregnancies in 80 women with f V Leiden we got the results which are shown in table 5. Stillbirths did not occur in any of these women. Protein C, protein S and AT III deficiencies were not found in any of 8 women with abortions. Antiphospholipid syndrome was diagnosed in one woman with recurrent abortions (positivity of LA and ACA). Frequency of women with abortion in this group is 10% (4%-19%). Frequency of abortions is 8% (4%-13%).

3. The control group

The results in cohort of women without f V Leiden are shown in table 6. Stillbirths did not occur in any of these women. Protein C, protein S, AT III deficiencies were not found in any of 4 women with spontaneous abortions either. Frequency of women with abortion in this group is 9% (2%-21%), and frequency of abortions is 6% (2%-12%). Using Fisher's exact test we have not found statistical difference ($p=0,1$) between frequency of women with abortions in the retrospective group and in the control group. We have not proven statistical difference ($p=0,63$) between frequency of abortions in women in these groups either.

Tab. 1: Characteristics of females with spontaneous or recurrent fetal loss.

Number (No.) of women (w.)	30
Mean age (yrs.)	28
Age range (yrs.)	21-41
No. of pregnancies	43
Mean age of pregnant w. (yrs)	26
Age range of pregnant w. (yrs.)	21-41

Tab. 2: Characteristics of females with APC-R and f V Leiden.

No. of w.	80
Mean age (yrs.)	43
Age range (yrs.)	23-70
No. of pregnancies	172
Mean age of pregnant w. (yrs.)	25
Age range of pregnant w. (yrs.)	18-37

Tab. 3: Characteristics of females without APC-R and f V Leiden.

No. of w.	45
Mean age (yrs.)	43
Age range (yrs.)	24-72
No. of pregnancies	104
Mean age of pregnant w. (yrs.)	24
Age range of pregnant w. (yrs.)	19-38

Tab. 4: The results in the prospective group.

No. of w. with 1 spontaneous abortion	21
No. of w. with 2 spontaneous abortions	6
No. of w. with recurrent abortions	3
Mean week of abortion	10
Week range of spontaneous abortion	7-27
No. of w. with APC-R	2
No. of w. with f V Leiden	1

Tab. 5: Frequency of abortions in women with APC-R and f V Leiden.

No. of w. with 1 spontaneous abortion	4
No. of w. with 2 spontaneous abortions	3
No. of w. with recurrent abortions	1
Mean week of abortion	10,5
Week range of spontaneous abortion	7-18

Tab. 6: Frequency of abortions in women without APC-R and f V Leiden.

No. of w. with 1 spontaneous abortion	3
No. of w. with 2 spontaneous abortions	0
No. of w. with recurrent abortions	1
Mean week of abortions	11
Week range of spontaneous abortion	7-18

Discussion

Since 1996 several reports have been published about the relationship between f V Leiden and fetal loss. These studies assessed either the association of f V Leiden with recurrent fetal loss or the association with miscarriages and stillbirths. EPCOT study (European Prospective Cohort on Thrombophilia) is the largest study which analyzed the risk of fetal loss in women with known thrombophilia. Researchers found that the odds ratios were 3,6 (95% CI 1,4-9,4) for stillbirths and 1,3 (95% CI 0,94-1,71) for miscarriages. The odds ratios were 2,0 (0,5-1,77) for stillbirths and

0,9 (0,5-1,5) for miscarriages in women-carriers of f V Leiden (21). On the other in Berkane's study, f V Leiden was not found to be a common risk for stillbirths (1). The most recent prospective study performed in Sweden, comprising 2480 women with APC-R in early pregnancy, elucidated its obstetrics consequences. The presence of APC-R was unrelated to adverse pregnancy outcome apart from an 8 fold increased risk of VTE (18).

Three recent case control studies documented a significantly increased prevalence of factor V Leiden mutation in women with recurrent fetal loss (22,11,5). The increased prevalence was not found in study done by Kotwal (17). The discrepancies in the results may be explained by differences in selection criteria, including the ethnic origin of the study populations. Other potential causes for recurrent fetal loss, like chromosomal abnormalities, autoimmune disorders, endocrinologic diseases, infections and anatomic abnormalities should be eliminated as well. F V Leiden is a mild risk factor for thrombosis and is also a mild risk factor for recurrent pregnancy loss (3) but the majority of women who are carriers of f V Leiden will not experience a recurrent fetal loss.

Conclusion

Resistance to activated protein C and f V Leiden were not the risk factors for miscarriage and stillbirth in our study group. They have not been found to be the risk factors for recurrent fetal loss either. Prophylactic anticoagulant treatment is not indicated in women with APC-R and f V Leiden and with the history of fetal loss in their next pregnancies.

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