Combined inherited thrombophilia and adverse pregnancy outcome

G. Androutsopoulos[1], A. Mougiou[2], M. Karakantza[2], G. Sakellaropoulos[3], G. Kourounis[1], G. Decavalas[1]

[1]Department of Obstetrics and Gynaecology, [2]Department of Internal Medicine, Division of Hematology, [3]Medical Physics Department, University of Patras, Medical School, Rion (Greece)

Summary

Inherited thrombophilia has been suggested as a possible condition of increased susceptibility to adverse pregnancy outcomes. In our prospective study, we investigated the association between combined inherited thrombophilia and adverse pregnancy outcome in the South-Western Greek population.

Three hundred and ninety-six healthy Greek women with spontaneous pregnancies were investigated for combinations of the three commonest thrombophilic mutations (Factor II G20210A, Factor V Leiden and MTHFR C677T) and followed for adverse pregnancy outcomes. Statistical analysis was performed by Pearson's chi-square test.

Four women (1%) had the FV Leiden/MTHFR T677T double genotype and two women (0.5%) had the FII G20210A/MTHFR T677T double genotype. Although the small number of cases of combined inherited thrombophilia, it seems that the presence of FV Leiden/MTHFR T677T double genotype increases the risk for placental abruption.

Key words: Factor II G20210A; Factor V Leiden; MTHFR C677T; Combined inherited thrombophilia; Pregnancy outcome.

Introduction

Inherited thrombophilia, the principal risk factor for maternal thromboembolism, has been suggested as a possible condition of increased susceptibility to adverse pregnancy outcomes. Successful pregnancy outcome is dependent on the development and maintenance of adequate placental circulation. Abnormalities of placental vasculature may result in a number of gestational pathologies, including early and late pregnancy loss, placental abruption, intrauterine growth retardation (IUGR), intrauterine fetal death (IUFD) and preeclampsia (PE) [1]. All these outcomes complicate up to 0.2-3% of pregnancies and are the leading causes of perinatal morbidity and mortality [2].

The presence of acquired or inherited thrombophilic factors increases the risk of thromboembolic disease in the mother, while these factors have also been implicated in the development of gestational complications associated to placental vasculopathy [3].

In our prospective study, we investigated the association between combined inherited thrombophilia and adverse pregnancy outcome in the South-Western Greek population.

Material and Methods

Between January 2004 and January 2006, 396 women with spontaneous pregnancies were referred to the Outpatient Clinic of the Obstetrics and Gynaecology Department of the University of Patras Medical School. All women were investigated for the three commonest thrombophilic mutations (Factor II G20210A, Factor V Leiden and MTHFR C677T) and followed for adverse pregnancy outcome. Women with known inherited thrombophilia, pre-existing antiphospholipid syndrome (APS) and antiphospholipid antibodies were excluded from the study. The study was approved by the Ethical Committee of the hospital. Written informed consent was obtained from each woman. All women were started on supplements of folate and iron the 13th week of gestation.

Blood samples in EDTA were collected from all women on their first visit to the Outpatient Clinic (6-8 weeks of gestation). Molecular diagnosis was performed after DNA isolation, polymerase chain reaction (PCR) amplification, and hybridization of amplification products using allele specific oligonucleotide probes for the detection of Factor II G20210A, Factor V G506A and MTHFR C677T normal and mutated alleles (Thrombophilia Gene Mutation Assay Kit, Vienna Lab, Austria).

Adverse pregnancy outcomes were considered for all fetal losses (spontaneous abortion and intrauterine death), as well as gestational complications with fetomaternal circulatory disturbances (placental abruption, intrauterine growth retardation and preeclampsia).

Intrauterine death (IUD) was defined as fetal loss after 24 weeks' gestation.

Placental abruption (PA) was defined as the separation of the placenta from its site of implantation before the delivery of the fetus [4].

Intrauterine growth retardation (IUGR) was defined as a birth weight below the 5th percentile for gestational age [5].

Preeclampsia (PE) was defined by a blood pressure above 140/90 mmHg after 20 weeks' gestation, proteinuria > 300 mg/24 hours or persistent 30 mg/dl (1+ dipstick) in random urine samples. The term severe preeclampsia is used when a blood pressure above 160/110 mmHg is recorded at least six hours apart, and proteinuria of more than 5 g during 24 h occurs [6].

Statistical analyses were performed using the SPSS-12 for Windows. The chi-square test was used to assess the association between categoric variables.

Revised manuscript accepted for publication April 23, 2007

Results

From the 396 women included in the study, four women (1%) had the FV Leiden / MTHFR T677T double genotype and two women (0.5%) had the FII G20210A/MTHFR T677T double genotype. The demographics of women with double thrombophilic genotypes are shown in Table 1.

From the six women with double thrombophilic genotypes, three (50%) developed gestational complications during the follow-up of their current pregnancy.

None of the women with double thrombophilic genotypes developed thromboembolic complications during pregnancy.

Table 1. — *Women's demographics (n = 6).*

		Women with complications (n = 3)	Women without complications (n = 39
No. of pregnancies	1 pregnancy	1 (33.3%)	1 (33.3%)
	≥ 2 pregnancies	2 (66.7%)	2 (66.7%)
Age of women	< 25	0 (0%)	0 (0%)
	25-35	1 (33.3%)	1 (33.3%)
	> 35	2 (66.7%)	2 (66.7%)
Complications in previous pregnancies	No	2 (66.7%)	2 (66.7%)
	Yes	1 (33.3%)	1 (33.3%)
Smoking	No	1 (33.3%)	1 (33.3%)
	Yes	2 (66.7%)	2 (66.7%)
Diabetes mellitus	No	3 (100%)	3 (100%)
	Yes	0 (0%)	0 (0%)
Gestational diabetes	No	3 (100%)	3 (100%)
	Yes	(0%)	(0%)

Discussion

Normal pregnancy is an acquired thrombophilic condition in which marked changes in hemostasis take place. The net effect of these pregnancy-induced changes is to promote clot formation, extension and stability. These alterations protect pregnant women from severe haemorrhage during delivery, but they also predispose them to thromboembolic complications [7].

The coexistence of other acquired or inherited thrombophilic factors increases the risk of thromboembolic disease in the mother, while these factors have also been implicated in the development of gestational complications associated with placental vasculopathy [3].

The pathogenetic mechanisms responsible for placental vascular pathologies in women with thrombophilia have not been fully elucidated. It is yet unknown why only some women with thrombophilia express vascular gestational pathologies, while others do not. It is possible that this may relate to local factors affecting coagulation, fibrinolysis and vascular tone at the level of the placental vessels [8].

Coinheritance of multiple thrombophilic defects are significantly associated with increased risk of venous thrombosis [9-11]. The highest risk is exerted by the simultaneous presence of FV Leiden and FII G20210A (OR, 85.198) [11, 12]. Similarly, MTHFR 677T/T increases the risk associated with FV Leiden or with FII G20210A [13]. The simultaneous presence of FV Leiden and MTHFR T677T may be manifested by severe thrombotic events [9, 14]. In our study none of the women with double thrombophilic genotypes developed thromboembolic complications during pregnancy.

The large European Prospective Cohort on Thrombophilia study (EPCOT) revealed that FV Leiden combined with other thrombophilic defects had an increased risk for fetal loss [15]. Also the highest odds ratio for stillbirth (OR, 14.3; 95% CI, 2.4-86) was documented in patients with combined thrombophilic defects [15]. In another study, combined thrombophilic defects were documented in 21% of women with pregnancy loss compared with 5.5% of control patients [16]. Idiopathic recurrent miscarriage appears to increase in frequency when two or more concomitant thrombophilic defects are present [15]. In our study one woman (25%) with the FV Leiden/MTHFR T677T double genotype had a spontaneous abortion.

In a recent study a strong association between FV Leiden and FII G20210A mutation was demonstrated with primary miscarriages [17], confirming previous findings [18, 19]. In our study FV Leiden/FII G20210A double genotype was not detected.

When thrombophilic factors were considered in combination, the MTHFR mutation seemed to significantly increase the risk of pregnancy wastage [18, 20]. Similarly, combinations of thrombophilic states may further increase the risk for recurrent fetal loss [9]. In the Nimes Obstetricians and Haematologists Study 5 (NOHA5), placental pathologic vascular findings were documented in 88% of women with combined thrombophilia and in 100% of those with a combination of any thrombophilia and MTHFR T677T [21]. In our study three women (75%) with FV Leiden/MTHFR T677T double genotype developed pregnancy complications (1 spontaneous abortion and 2 placental abruptions).

Explanations offered for the increased risk of pregnancy loss include both thrombosis of placental vessels and embryotoxicity. Methylenetetrahydrofolate reductase catalyzes remethylation of homocysteine to methionine. Homozygous MTHFR C677T gene mutation has been associated with elevated levels of homocysteine and has been identified as a risk factor for thrombosis [22]. The embryotoxic effects have been postulated to be related to the influence of homocysteine on the proliferation of rapidly dividing embryonic cells [23].

Multiple thrombophilic factors carry a major additional risk for adverse maternal and fetal outcomes and correlate well with placental maladaptation as indicated by uterine Doppler velocimetry and 24 h blood pressure monitoring. A reason for this finding may be that the low pressure intervillous blood flow in the presence of a maternal hypercoagulable state might trigger fibrin deposition in the placenta and cause placental infarcts that might incite development of severe disease [20].

In our study two women (50%) with FV Leiden/MTHFR T677T double genotypes developed placental abruption. It seems that the presence of FV Leiden/MTHFR T677T double genotype increases the risk for placental abruption.

The main limitation of our study was the small number of cases of combined inherited thrombophilia. These data require confirmation in large, especially prospective, studies.

Conclusion

Despite the small number of cases of combined inherited thrombophilia, it seems that the presence of FV Leiden/MTHFR T677T double genotype increases the risk for placental abruption.

References

[1] Salafia C.M., Minior V.K., Pezzullo J.C., Popek E.J., Rosenkrantz T.S., Vintzileos A.M.: "Intrauterine growth restriction in infants of less than thirty-two weeks' gestation: associated placental pathologic features". *Am. J. Obstet. Gynecol.,* 1995, *173,* 1049.

[2] Abbate R., Sofi F., Gensini F., Fatini C., Sticchi E., Fedi S.: "Thrombophilias as risk factors for disorders of pregnancy and fetal damage". *Pathophysiol Haemost. Thromb.,* 2002, *32,* 318.

[3] Greer I.A.: "Thrombophilia: implications for pregnancy outcome". *Thromb. Res.,* 2003, *109,* 73.

[4] Cunningham G., Leveno K., Bloom S., Hauth J., Gilstrap III L., Wenstrom K.: Williams Obstetrics 22nd edn., New York, McGraw-Hill, 2005, 811.

[5] Seeds J.W.: "Impaired fetal growth: definition and clinical diagnosis". *Obstet. Gynecol.*, 1984, *64*, 303.

[6] Cunningham G., Leveno K., Bloom S., Hauth J., Gilstrap III L., Wenstrom K.: Williams Obstetrics, 22nd edn., New York, McGraw-Hill, 2005, 762.

[7] Bremme K.A.: "Haemostatic changes in pregnancy". *Best. Pract. Res. Clin. Haematol.,* 2003, *16,* 153.

[8] Brenner B.: "Thrombophilia and pregnancy loss". *Thromb. Res.,* 2002, *108,* 197.

[9] Mandel H., Brenner B., Berant M., Rosenberg N., Lanir N., Jakobs C. *et al.*: "Coexistence of hereditary homocysteinuria and factor V Leiden-effect on thrombosis". *N. Engl. J. Med.,* 1996, *334,* 763.

[10] Brenner B., Zivelin A., Lanir N., Greengard J.S., Griffin J.H., Seligsohn U.: "Venous thromboembolism associated with double heterozygosity for R506Q mutation of factor V and for T298M mutation of protein C in a large family of a previously described homozygous protein C deficient newborn with massive thrombosis". *Blood,* 1996, *88,* 877.

[11] Almawi W.Y., Tamim H., Kreidy R., Timson G., Rahal E., Nabulsi M. *et al.*: "A case control study on the contribution of factor V-Leiden, prothrombin G20210A, and MTHFR C677T mutations to the genetic susceptibility of deep venous thrombosis". *J. Thromb. Thrombolysis*, 2005, *19*, 189.

[12] Martinelli I., Taioli E., Cetin I., Marinoni A., Gerosa S., Villa M.V. *et al.*: "Mutations in coagulation factors in women with unexplained late fetal loss". *N. Engl. J. Med.,* 2000, *343*, 1015.

[13] Fujimura H., Kawasaki T., Sakata T., Ariyoshi H., Kato H., Monden M. *et al.*: "Common C677T polymorphism in the methylenetetrahydrofolate reductase gene increases the risk for deep vein thrombosis in patients". *Thromb. Res.*, 2000, *98,* 1.

[14] Ridker P.M., Hennekens C.H., Selhub J., Miletich J.P., Malinow M.R., Stampfer M.J.: "Interrelation of hyperhomocysteinemia, factor V Leiden, and risk of future venous thromboembolism". *Circulation,* 1997, *95*, 1777.

[15] Preston F.E., Rosendaal F.R., Walker I.D., Briet E., Berntorp E., Conard J. *et al.*: "Increased fetal loss in women with heritable thrombophilia". *Lancet,* 1996, *348,* 913.

[16] Sarig G., Younis J.S., Hoffman R., Lanir N., Blumenfeld Z., Brenner B.: "Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage". *Fertil. Steril.,* 2002, *77*, 342.

[17] Finan R.R., Tamim H., Ameen G., Sharida H.E., Rashid M., Almawi W.Y.: "Prevalence of factor V G1691A (factor V-Leiden) and prothrombin G20210A gene mutations in a recurrent miscarriage population". *Am. J. Hematol.,* 2002, *71,* 300.

[18] Brenner B., Sarig G., Weiner Z., Younis J., Blumenfeld Z., Lanir N.: "Thrombophilic polymorphisms are common in women with fetal loss without apparent cause". *Thromb. Haemost.,* 1999, *82*, 6.

[19] Souza S.S., Ferriani R.A., Pontes A.G., Zago M.A., Franco R.F.: "Factor V Leiden and factor II G20210A mutations in patients with recurrent abortion". *Hum. Reprod.,* 1999, *14,* 2448.

[20] Tranquilli A.L., Giannubilo S.R., Dell'Uomo B., Grandone E.: "Adverse pregnancy outcomes are associated with multiple maternal thrombophilic factors". *Eur. J. Obstet. Gynecol. Reprod. Biol.,* 2004, *117,* 144.

[21] Gris J.C., Quere I., Monpeyroux F., Mercier E., Ripart-Neveu S., Tailland M.L. *et al.*: "Case control study of the frequency of thrombophiilc disorders in couples with late foetal loss and no thrombotic antecedent - the Nimes Obstetricians and Haematologists Study 5 (NOHA5)". *Thromb. Haemost.,* 1999, *81,* 891.

[22] Kluijtmans L.A., van den Heuvel L.P., Boers G.H., Frosst P., Stevens E.M., van Oost B.A. *et al.*: "Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease". *Am. J. Hum. Genet.,* 1996, *58,* 35.

[23] Hasbargen U.., Lohse P., Thaler C.J.: "The number of dichorionic twin pregnancies is reduced by the common MTHFR 677C>T mutation". *Hum. Reprod.*, 2000, *15,* 2659.

Address reprint requests to:
G. ANDROUTSOPOULOS, M.D.
Anaxagora, 45
Ag. Parskeui
15343 Greece