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Unexplained infertility: Association with inherited thrombophilia

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ABSTRACT

Introduction: Unexplained infertility represents one of the most common diagnoses in fertility care. Attention is being paid to the association between inherited thrombophilia and infertility causes. In this study we investigated the prevalence of inherited thrombophilia according to infertility causes.
Materials and methods: We studied Prothrombin gene G20210A mutation, Factor V Leiden, deficiencies in protein S and C and antithrombin in 930 Caucasian infertile women referred to Fertility Center of the Department of Sciences for Woman and Child's Health, University of Florence, of whom 230 with unexplained, 195 female and 283 male infertility, and in 240 women who have conceived naturally without hormonal stimulation therapy.
Results: A significant relationship between inherited thrombophilia [OR 95%CI 1.97 (1.05-3.68), p = 0.03] and unexplained infertility was observed, whereas no association between thrombophilia and female and male infertility was found. Significantly higher prevalence of prothrombin gene mutation in unexplained infertile women in comparison to that observed in fertile women was observed (5.7% *vs* 2.1% p = 0.04); the prevalence of the other thrombophilia determinants was higher, even if not significantly, in the unexplained infertile group.
Conclusions: This study demonstrates the relationship between inherited thrombophilia and unexplained infertility, thus suggesting the contribution of genetic components in modulating unexplained infertility, behind anovulation, male and tubal factor.

Introduction

Inherited thrombophilia represents a risk factor for vascular disease and thrombophilia-associated risk may increase in women undergoing assisted reproductive procedures, in whom the use of exogenous hormones may affect both coagulation and fibrinolytic system [1], thus inducing a prothrombotic phenotype.

Infertility affects 13% to 15% of couples worldwide [2], and unexplained infertility represents one of the most common diagnoses in fertility care [3]. Possible underlying mechanisms responsible for unexplained infertility have been proposed, and this topic still remains object of interest [4].

Attention is being paid to the association between inherited thrombophilia and infertility, and few studies, performed on small series of subjects, investigating the relationship between thrombophilia and unexplained infertility, are available [5,6].

Most information concerning the influence of inherited thrombophilia on fertility derives from studies investigating the relationship between thrombophilia and implantation failure with conflicting results [7–11].

Therefore, due to the poor information available concerning the relationship between inherited thrombophilia and infertility, we performed a case–control study in order to evaluate the prevalence of inherited thrombophilia according to different infertility causes in a large sample of Italian women affected by couple infertility.

Materials and methods

Study population

Consecutive women (n = 1802), median age 35 yrs, ranging from 18 to 46 yrs consulting for couple infertility, defined as the inability to conceive after 1 year of regular unprotected intercourse, from 2007 to 2010 to the Fertility Center of the Department of Sciences for Woman and Child's Health, University of Florence, were enrolled.

Abbreviations: PC, protein C; PS, protein S; AT, antithrombin; FVL, Factor V Leiden; PT, prothrombin gene.

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All couples went through a standard fertility work-up, consisting of: complete anamnestical evaluation of both partners, karyotype analysis, semen analysis, cycle monitoring, including mid-luteal progesterone, and tubal work-up, including Chlamydia antibody test (CAT), hysterosalpingography (HSG) or laparoscopy with dye test. When the results of the standard infertility evaluation were normal, diagnosis of unexplained infertility was assigned, according to the Practice Committee of the American Society for Reproductive Medicine 2006 [12].

Out of the 1802 women, 607 (33.7%) women did not complete clinical and laboratory *iter*, 40 (2.2%) refused to assent with the genetic analysis, 19 (1.1%) because of familial or personal history of vascular disease, and 121 (6.7%) were excluded as no-Caucasian. Eighty-five (4.7%) out of 1802 women older than 40 yrs have been excluded, due to the higher likelihood of age-related infertility, in order to avoid an incorrect assessment of infertility cause.

Among remained infertile women (n = 930), 230 showed unexplained infertility, 195 showed female infertility (tubal abnormalities, endometriosis, and anovulatory cycles), 283 showed male infertility, and remained women exhibited more than one cause of infertility. All 930 infertile women had primary infertility (i.e. never previously conceived, spontaneously or after assisted procedure), independently of infertility cause.

All 930 women were analyzed for the presence of congenital inherited thrombophilia (factor V Leiden mutation, prothrombin gene G20210A polymorphism, and deficiencies in protein S and C and AT). Every woman in the study groups was taking folic acid in order to plan pregnancy, thus we could not evaluate the homocysteinemia as marker of prothrombotic status. Women underwent blood draw before starting any hormonal treatment in order to avoid hormonal influence on anticoagulant proteins.

As control group we enrolled 240 Caucasian apparently healthy women, comparable for age, who had conceived naturally without hormonal stimulation therapy, and gave birth to healthy infants, and without personal history of vascular events. Informed written consent was obtained from all women, and the study was approved by the Institutional Review Board of the Azienda Ospedaliero-Universitaria, Careggi.

Statistical analysis

Statistical analysis was performed by using the SPSS (Statistical Package for Social Sciences, Chicago, USA) software for Windows (Version 11.5).

Assuming a 7% prevalence of at least 1 inherited thrombophilia determinant in fertile women, and a prevalence of 16% of at least 1 inherited thrombophilia determinant in unexplained infertile women reported in literature [13], at least 197 subjects should be included in the study, in order to achieve a statistical power of 80% ($\beta = 0.8$) and significance value of 0.05 (α).

Categorical variables were expressed as frequencies and percentages. As deficiency of anticoagulant proteins (protein C, S and antithrombin) represents a rare condition, we considered the combination of these defects as a single variable in the statistical analysis. The χ^2 test was used to test for proportions between analysis groups. Odds ratio (OR) with 95% confidence interval (CI) was determined. A p-value <0.05 was considered to indicate statistical significance.

Results

Demographic, clinical and thrombophilic characteristics of study population are reported in Table 1.

Ninety-eight out of 930 infertile women (10.5%) had at least one of the inherited determinants of thrombophilia and 3 (0.3%) women had the contemporary presence of two thrombophilic defects (protein S/prothrombin G20210A polymorphism, and factor V Leiden/prothrombin G20210A polymorphism). Seventeen out of 240 (7.1%) fertile controls had at least one of the inherited determinants of thrombophilia, and no contemporary presence of more than one thrombophilic defect was found. A higher prevalence, even if not significant, of prothrombin 20210A variant and anticoagulant proteins in comparison to that observed in fertile women was observed (Table 1).

Table 1
Demographic, clinical and thrombophilic characteristics of study population.

Variables	Infertile women n = 930	Fertile women n = 240	P value
Age*	35 (20–40)	35 (21–40)	0.1
Smoking habit, n (%)	198 (21.3)	41 (17.1)	0.1
BMI > 25Kg/m^2, n (%)	162 (17.4)	40 (16.7)	0.8
Infertility years*	4 (2–8)	-	-
Infertility cause		-	-
Male factor, n(%)	283 (30.4)	-	-
Tuboperitoneal factor, n(%)	144 (15.5)	-	-
Anovulation, n(%)	51 (5.5)	-	-
Unexplained, n(%)	230 (24.7)	-	-
Mixed causes, n(%)	222 (23.9)	-	-
^Thrombophilia, n (%)	98 (10.5)	17 (7.1)	0.1
PC + PS + AT deficiency, n (%)	22 (2.4)	3 (1.2)	0.3
Factor V Leiden, n (%)	36 (3.9)	9 (3.8)	0.9
Prothrombin G20210A, n (%)	43 (4.6)	5 (2.1)	0.07

* median (range);^3 out of 98 women exhibited more than one inherited thrombophilic defect.

When the 930 women undergoing assisted reproductive procedures were sub-grouped according to infertility cause, we found unexplained infertility in 230 (group A), female infertility in 195 (tubal abnormalities, endometriosis, and anovulatory cycles) (group B), and male infertility in 283 (group C); more than one cause of infertility was recognized in the remaining women.

A significantly higher prevalence of thrombophilia in unexplained infertility (13.0%), but not in female (9.7%) and male (9.2%) infertility, than in fertile (7.1%) women was observed (Table 2), (Multivariate regression logistic analysis: model 1: OR = 2.70 CI95% 1.18-6.20, p = 0.02, adjusted for age; model 2: OR = 3.35 CI95% 1.59-7.05, p = 0.001, adjusted for age, BMI and smoking habit).No difference in thrombophilia prevalence among infertility causes (group A vs B vs C) has been observed (Table 2). Moreover, as each thrombophilia determinant is considered, a significantly higher prevalence of prothrombin gene mutation in the unexplained group in comparison to that observed in fertile women was observed (OR = 2.82 CI 95% 1.02-8.03, p = 0.04). The prevalence of factor V Leiden and anticoagulant proteins deficiencies was higher, but not significantly, in unexplained infertility group in comparison to that observed in fertile women (Table 2).

Discussion

The present study, which investigated the relationship between inherited thrombophilia and different causes of infertility, demonstrated higher prevalence of inherited thrombophilia, and in particular of prothrombin gene mutation, in women with unexplained infertility, thus suggesting the involvement of genetic components in modulating infertility, behind anovulation, male and tubal factor.

Unexplained infertility is idiopathic in the sense that its cause remains unknown [according to the Practice Committee of the American Society for Reproductive Medicine 2006 [12]], and potential mechanisms possibly responsible for unexplained infertility still remain object of interest.

Intriguing information from literature reported a role for factor V Leiden in inferring selective advantage in male fertility [14], possibly due to a direct function of the factor V protein in spermatogenesis. The high frequency of factor V Leiden could result in a balance between allele loss, through thrombosis and pregnancy loss, and allele