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ORIGINAL ARTICLE

Elevated plasma levels of factor VIII in women with early recurrent miscarriage

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Summary. *Aims*: Inherited and acquired thrombophilia have been found to be associated with recurrent pregnancy loss. This paper examines whether or not elevated factor (F)VIII:C plasma levels, which have been demonstrated to be an independent risk factor for venous thromboembolism, are a risk factor for early recurrent miscarriages also. *Patients and methods*: Consecutive women referred to our clinic with a history of early recurrent abortion (at least three pregnancy losses before week 13 of gestation) were eligible for the study. Exclusion criteria were endocrine, immunological, anatomical and genetic causes of embryo demise, as well as any thrombophilic abnormality, either congenital or acquired, or a personal or familial history of venous thromboembolism. FVIII:C plasma levels were determined in 51 cases and in 51 controls matched for age, ethnicity and blood group. *Results*: The mean FVIII:C level in the control subjects was 106.8 IU dL^{-1}, compared with 128.2 IU dL^{-1} in the patients group ($P = 0.0002$). Thirteen (25.5%) of the 51 patients had FVIII:C values exceeding the 90th centile of the control population (145 IU dL^{-1}), compared with four subjects in the control group ($\chi^2 = 4.52$; $P = 0.033$; odds ratio =4.02, 95% confidence interval 1.09, 16.05). No cases with increase in FVIII:C levels attributable to an acute-phase reaction, as assessed by C-reactive protein plasma concentration, were found. *Conclusions*: We found FVIII:C levels significantly higher in women with early recurrent miscarriage compared with controls. This finding suggests a possible association between this thrombophilic condition and early reproductive failures.

Keywords: early recurrent abortion, factor VIII, thrombophilia.

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Introduction

Recurrent miscarriage, defined as three or more spontaneous fetal losses prior to the 20th gestational week, is a common health problem, as it affects 1–2% of couples attempting to reproduce [1,2]. Known causes or associations of recurrent miscarriage are endocrine, immunological, anatomical and genetic, although about 50% of cases are classified as idiopathic [3]. In recent years attention has been drawn to the possible association of both early and late pregnancy loss with either acquired or inherited thrombophilic defects predisposing to the development of deep vein thrombosis (DVT), namely factor (F)V R506Q (FV Leiden) and prothrombin gene G20210A mutations, hyperhomocysteinemia and antiphospholipid antibodies [4–13]. Recently, a growing literature has suggested that elevated levels of FVIII:C may represent a risk factor for venous thromboembolism (VTE). Koster *et al*. in their analysis of the Leiden Thrombophilia Study reported that FVIII clotting activity was associated as a continuous variable with VTE [14]. They found that compared with control subjects the adjusted odds ratio for FVIII:C levels above 90th centile, thus exceeding the value of 150 IU dL^{-1}, was 4.8-fold [14]. In additional studies it was demonstrated that the increase in FVIII:C levels is consistent over time in thromboembolic patients and it is not attributable to an acute-phase reaction, as assessed by C-reactive protein (CRP) plasma concentration [15,16]. Furthermore, a gradual dose–response relationship between FVIII:C levels and the risk of venous thrombosis was demonstrated [17], as well as an increased risk of recurrent VTE for patients with elevated FVIII:C plasma levels [18].

To the best of our knowledge, however, no studies addressed the issue of the association between FVIII:C levels and the risk of reproductive failure. In this prospective case–control study we therefore tried to determine whether or not elevated FVIII:C plasma levels are to be considered a risk factor for early recurrent miscarriage.

Patients and methods

Consecutive women referred to our clinic between January 1999 and July 2002 with a history of early recurrent abortion

were eligible for the study. In order to obtain a well-selected population of very early aborters, we decided to evaluate only women with at least three pregnancy losses before week 13 of gestation. All patients had a thorough investigation for known potential causes of embryo demise, which included: fasting glucose, baseline follicle-stimulating hormone (FSH), lutenizing hormone (LH) and estradiol levels on day 3 of a natural cycle, thyroid-stimulating hormone (TSH) and prolactin levels and antinuclear factor, vaginal and cervical swabs. Hysterosalpingography and/or hysteroscopy as well as transvaginal ultrasound were routinely performed to rule out uterine malformations. A thorough complete screening for either congenital or acquired thrombophilia was performed, including search for antiphospholipid antibodies, Activated Protein C (APC) resistance, FV R506Q and prothrombin G20210A mutations, protein C, S and antithrombin III (ATIII) levels and fasting homocysteine level measured by fluorescence polarization immunoassay (FPIA), as previously described [19–24].

Exclusion criteria were: metabolic or immunological causes of fetal demise; abnormal karyotype in either partner of the couple; documented first-trimester preclinical and blighted ovum abortions; history of at least one pregnancy loss resulting from a documented embryo/fetal malformation or abnormal placental insertion or uterine malformation. Women were also excluded if they had any thrombophilic abnormality, either congenital or acquired, as well as a personal or familial history of VTE.

The control population consisted of women living in the same Modena area, recruited in the same 4-year period having had at least one uneventful pregnancy giving birth to a normal weight, term baby and no history of fetal loss. Cases and controls were matched for age, ethnicity and blood group and were examined at least 60 days after their last pregnancy. None was taking oral contraceptives, or was lactating. After obtaining informed consent, 20 mL of blood were collected by venipuncture with minimal stasis using a 19-G butterfly needle into 0.109 mol L^{-1} trisodium citrate, nine parts blood to one part anticoagulant. Platelet-poor plasma was prepared by double centrifugation of samples at 2700 *g* at room temperature for 20 min and stored at −70 °C. FVIII:C level was measured by one-stage clotting assays with FVIII-deficient plasma and automated activated partial thromboplastin time (Dade Behring, Deerfield, IL, USA) as previously described [16]. The FVIII:C plasma concentration was expressed in IU dL^{-1}. In order to minimize the effects of fluctuations in FVIII:C levels, blood samples were collected in duplicate for each patient, at different times of the year as well as of the menstrual cycle. CRP levels were measured by CRP antigen–antibody reaction (Beckman Coulter Inc., Fullerton, CA, USA), in order to rule out an acute-phase reaction potentially interfering with FVIII:C levels. If CRP levels were above the normal range (5.0 μg L^{-1}), subjects were not included in the study.

All values are expressed as means (±SD). FVIII:C values are reported as the average of the two assays. Data were compared using the Social Science Software Statistical Package (SPSS Inc., Chicago, IL, USA). ANOVA was applied for continuous variables comparisons. χ^2 was used in the case of categorical variables. The 90th centile of control population distribution was chosen as cut-off value for FVIII:C levels.

Results

Twenty-four of the 75 consecutive women with early (before week 13) recurrent pregnancy loss were excluded: three showed APC resistance (two of them linked to FV R506Q heterozygous mutation, and the other one of the acquired type), 12 had embryos with congenital or genetic abnormalities, four had uterine or placental malformations, five had preclinical and blighted ovum abortions. All the 51 remaining women agreed to participate in the study. Nineteen patients were secondary aborters having had one or two (one case) successful pregnancies followed by repeated abortions. The mean age of patients (32.8 ± 5.3 years, range 20–44) and controls (32.2 ± 6.2 years, range 22–45) was comparable, as well as the proportion of non-Caucasian women (15% and 18%, respectively). Clinical features of cases and controls are reported in Table 1.

The mean (±SD) FVIII:C level in the control subjects was 106.8 ± 23.1 IU dL^{-1}, compared with 128.2 ± 32.9 IU dL^{-1} in the patients group ($F = 14.4$, $P = 0.0002$). According to the distribution in controls, the 90th percentile of the FVIII:C was 145.0 IU dL^{-1} (Fig. 1). Thirteen (25.5%) of the 51 patients had values that exceeded such a cut-off point, compared with four subjects in the control group ($\chi^2 = 4.52$; $P = 0.033$; odds ratio =4.02, 95% confidence interval 1.09, 16.05). Once evaluated separately, the distribution of FVIII:C value of the first and the second sampling allows similar results. Indeed, 11 of the cases remained outside over the 90th centile in both series.

No relationship was observed between parity and elevated levels of FVIII:C. Indeed, nine (28.1%) of the 32 patients who had no successful pregnancies had FVIII:C levels above the cut-

Table 1 Clinical features of patients and controls

	Cases ($n = 51$)	Controls ($n = 51$)	*P*
Age (years)	32.8 ± 5.3 (20–44)	32.2 ± 6.2 (22–45)	ns
Caucasian (*n*)	8	9	ns
Age at first pregnancy (years)	27.7 ± 5.0 (17–37)	30.1 ± 6.1 (19–41)	
Number of pregnancies, including abortions (median)	4	1	–
Gestational age (weeks) at abortion ($n = 179$)	9.3 ± 1.6	–	–
Time between last pregnancy and first blood sample (months)	3.4 ± 0.5 (2–9)	10.1 ± 4.9 (6–23)	0.001

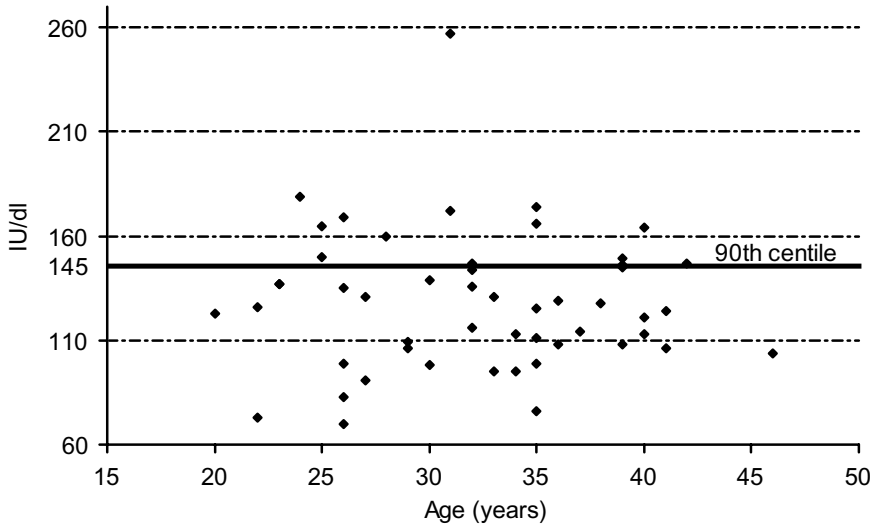

Fig. 1. Factor VIII:C levels of distribution.

off value of 145 IU dL^{-1}, compared with four (21.1%) of the 19 parous patients.

None of the three patients having an APC resistance showed FVIII:C levels above the cut-off value.

Discussion

The relationship between thrombophilic conditions and recurrent miscarriage is still an open issue. Several reports over the last years have suggested a potential association of first- as well as second-trimester recurrent pregnancy loss with either inherited or acquired thrombophilic defects leading to an increased risk of venous thrombosis, although some studies failed to find such an association [25,26]. Moreover, some authors found thrombophilia to be associated with late [27], some with early [28,29] and others with both early and late pregnancy loss [30]. A very recent meta-analysis indicates that first-trimester recurrent fetal loss is associated with FV Leiden, APC resistance and prothrombin G20210A mutation, although FV Leiden is even strongly associated with late fetal loss [13].

Recently, much attention has been drawn to elevated levels of coagulation FVIII:C as a risk factor for venous thromboembolism [14–18], but no studies addressed the issue if they are to be considered a risk factor for recurrent miscarriages also, as the above reported thrombophilic conditions.

In the present study we found that FVIII:C levels above the 90th centile of the control population are significantly associated with recurrent miscarriages before 13 weeks. Our data from a highly selected population of women with early recurrent miscarriages without other apparent causes are in keeping with some recent reports demonstrating that thrombophilia may contribute to the occurrence of early recurrent pregnancy loss as well [13,28–31]. The largest published study assessing the prevalence of FV Leiden and acquired APC resistance among more than 1000 women with recurrent miscarriage showed that acquired, but not congenital APC resistance is associated with both early and late reproductive failure [31], suggesting that a functional over-regulation of hemostatic balance could play a key role in determining placental vasculature thrombosis. Although the finding of placental vascular thrombosis is very rare in the products of early abortions, it could be hypothesized that hypercoagulable states can lead to some vascular impairment in the first phases of embryo implantation, also in absence of overt placental thrombosis. In physiological conditions the FVIII:C concentration rises as pregnancy advances, with a first increase between 11 and 16 weeks and a second, steeper one, between 16 and 18 weeks [32]. A gradual dose–response relationship between FVIII levels and the risk of thrombosis has been observed [14,17]. Therefore it can be argued that in predisposed women even a small increase in already elevated FVIII:C levels can attain a hypothetical threshold required for triggering the thrombotic event.

Some issues in such a pathophysiological picture are worth discussion. We choose the 90th centile of control population distribution as cut-off value for FVIII:C levels, according to previous studies [14,17]. However, in our study this cut-off corresponds to an absolute value of FVIII:C of 145 IU dL^{-1}, slightly lower than the value of 150 IU dL^{-1} previously reported as a risk factor for venous thromboembolism. We believe that many factors can explain this discrepancy. First, the mean age of our population was lower than that in the reported studies on venous thromboembolism (32 vs. 56 years), and it is well known that FVIII:C levels increase with age [33]. Second, a considerable proportion of our population (15%) was carrying the O blood group, which is associated with lower levels of both FVIII:C and von Willebrand factor [34]. Furthermore, given the physiological increase of FVIII:C levels throughout pregnancy, one might expect that elevated FVIII:C levels are an even stronger risk factor for late miscarriage. This important issue requires further and properly designed studies.

Many acquired variables, above all acute-phase reaction, may increase FVIII:C activity, and therefore only baseline persistently elevated FVIII:C levels are to be considered a risk factor for venous thromboembolism. In order to avoid inflammatory response as a confounding factor we measured CRP in all subjects , an established acute-phase marker. All CRP values

fell within the normal range, thus ruling out that in our case population elevated FVIII:C levels might be the result of an inflammatory status. We therefore demonstrated a significantly higher frequency of subjects with persistently elevated FVIII:C levels in women with early recurrent miscarriage. Given the variability of FVIII:C levels due to several pathophysiological factors, however, it remains to be established whether or not FVIII:C levels should be included in screening for thrombophilia in such patients.

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