Association between plasminogen activator inhibitor 1 gene mutation and different subgroups of recurrent miscarriage and implantation failure

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Abstract

Purpose To compare plasminogen activator inhibitor type1 (PAI-1) mutation rates in different groups of patients with the record of recurrent miscarriage (RM) or implantation failure (IF) with special emphasis on the number of missed pregnancies and/or implantation failures (RM $\geq$ 2, IF $\geq$ 2, RM + IF $\geq$ 2, RM $\geq$ 3, IF $\geq$ 3 and RM + IF $\geq$ 3).

Method Case-control study from PCR products and RFLP data of DNA from blood of patients who referred to the infertility clinic including 595 patients (421 RM $\geq$ 2, 119 IF $\geq$ 2 and 55 RM + IF $\geq$ 2) as the case groups and 100 healthy women as the control group.

Capsule The mutant (4G) allele of plasminogen activator inhibitor type 1 (PAI-1) is significantly increased in women with recurrent miscarriage (RM) and those with implantation failure (IF) compared to control women, suggesting a role for PAI-1 mutation in RM and IF.

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Results All six different subgroups of patients showed increased frequencies of the mutant allele (4G) in comparison to the control group ($p < 0.001$) suggesting a role for PAI-1 mutation in RM and IF.

Conclusions The different patient subgroups suffer similar rates of risk in developing RM and IF when compared to controls.

Keywords Implantation failure · Mutation · Plasminogen activator inhibitor type1 · Recurrent miscarriage · Thrombophilia

Introduction

Thrombophilic disorders have been proven to be risk factors in a vast number of women with recurrent miscarriage (RM) [1–3]. Hereditary thrombophilia is a group of genetic disorders of blood coagulation resulting in a hypercoagulable state, which in turn can result in abnormal placentation [4]. Thrombophilia has been associated with complications such as pre-eclampsia, intrauterine growth retardation, placental abruption and stillbirth [5].

Recurrent Implantation Failure (RIF) can be defined as the repeated lack of implantation after the transfer of embryos [6, 7]. A survey performed on 25 different populations confirmed that miscarriage frequently occurs in women willing to have children with an estimated overall median prevalence of 9 % [8]. RM is mostly defined as 2 or more spontaneous miscarriages [9, 10] and affects approximately 5 % of women at reproductive age [11], while others define it as 3 or more records of miscarriage, which affects 1 % of women at this age [12].

Although several causes for RM have been proposed, more than half of cases remain unexplained, which is a challenging

dilemma for both patients and physicians [11]. Various miscarriage rates in clinically detected pregnancies have been reported in different studies. RM occurs in early pregnancy prior to the 20th week of gestation [13]. The causes or clinical factors associated with RM are chromosomal abnormalities, anatomical alterations of the uterus, endocrinologic abnormalities, infections and autoimmune disorders [14]. In addition, convincing evidence that patients with inherited thrombophilic disorders have a higher risk for RM has prompted many researchers to study the frequency of homeostatic abnormalities in patients with RM [2, 14]. It is usually difficult to ascertain a biochemical or genetic risk factor in most RM patients. In a large study on more than 500 women with RM and infertility, 47 % showed a decreased response in venous occlusion test [15] which is used to measure fibrinolysis. In this regard, reduced fibrinolytic activity may be caused by increased plasminogen activator inhibitor (PAI) levels or decreased levels of tissue plasminogen activator (t-PA) [16].

Plasminogen activation is further regulated by the presence of very specific and rapid acting PAI molecules, which are also present in plasma [17]. The most important inhibitor of t-PA is PAI-1 [18]. Some studies have reported that PAI-1 and t-PA gene polymorphisms are possibly associated with hypofibrinolysis and thrombotic complications. In general, the plasminogen activator/plasmin, i.e. fibrinolytic system serves as one of the endogenous defense mechanisms for the prevention of intravascular thrombosis [19].

The concentrations of PAI-1 in plasma have been related to a common guanosine insertion/deletion gene polymorphism of 4G/5G located at the 675th base pair upstream of the start point of translation (−675 4G/5G). Homozygosity for the deletion genotype (4G/4G) has been associated with PAI-1 concentrations higher than those associated with the insertion genotype (5G/5G), causing reduced fibrinolytic activity [20, 21].

Although previous studies have investigated the association of the 4G/5G PAI-1 polymorphisms with infertility and RM [1, 22, 23], the present study has focused on the detailed comparison of this association in various types of pregnancy failures, including IF and RM groups who have experienced different numbers of missed pregnancies.

Material and methods

Subject selection

We included the data for 595 women (including 421 RM ≥ 2 , 119 IF ≥ 2 and 55 RM + IF ≥ 2) who were referred to Avicenna Infertility Clinic, Tehran, Iran during 2 years (between 2009 and 2011) as the case group. These women had an average age of 29.5 ± 5.3 , 33.5 ± 5 and 34.3 ± 5.6 years old for RM, IF and

RM + IF groups, respectively. Furthermore, the control group included 100 healthy women with an average age of 33 ± 3 years with at least two normal pregnancies with live births and no complicated pregnancies, miscarriages, still births, small for gestational age fetuses or pre-eclampsia.

Informed consent was obtained for all subjects who participated in the study. The women had been tested for PAI-1 (−675 4G/5G) gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [22]. The study was approved by the Ethics Committee of Avicenna Research Institute.

The individuals within the case group were divided into three groups namely those with miscarriage only (RM), those with implantation failure only (IF) and those having experienced both miscarriage and implantation failure (RM + IF). Each group was further subdivided into those with ≥ 2 and those with ≥ 3 incidences referred as ≥ 2 and ≥ 3 , respectively.

Statistical analysis

The genotype distributions, including homozygosity and heterozygosity proportions of the PAI-1 polymorphism among different groups of RM/IF and normal women were analyzed by Mann-Whitney *U* test using SPSS (Ver.13.0) software. Univariate logistic regression test was used for calculating odds ratios (ORs) as a measure of relative risk of RM/IF in association in the presence of the polymorphism.

Results

Using PCR- RFLP, DNA from patients in the six subgroups, 421(RM ≥ 2), 119(IF ≥ 2), 55 (RM + IF ≥ 2), 251(RM ≥ 3), 54(IF ≥ 3) and 40(RM + IF ≥ 3) (Table 1), were analyzed for carrying PAI-1 4G/5G Polymorphisms.

The percentages of homozygosity and heterozygosity for the 4G allele in all case subgroups were significantly higher than those in the controls. By contrast, the proportions of homozygosity for the 5G allele in all case subgroups were lower than those in normal individuals (Table 1).

Comparisons of the genetic distributions of PAI-1 polymorphisms between the 6 case groups and the control group, using Mann-Whitney *U* test, showed significant differences ($p < 0.001$) with higher frequencies of heterozygotic and homozygotic genotypes in the case groups (Table 1). Univariate regression analysis showed that homozygosity and heterozygosity for the 4G allele increased the risk of RM occurrence ($p < 0.001$, Table 2). But the odds ratios for all subgroups were roughly similar in homozygotic (47.81, 49.26, 44, 51.84, 44 and 55.38) and heterozygotic (4.33, 3.86, 3.85, 4.34, 3.7 and 3.49) polymorphisms in RM

Table 1 Comparison of the PAI-1 polymorphism in case and control groups

Genotype	Control (<i>n</i> =100)	Case (<i>n</i> ≥2=595 and <i>n</i> ≥3=345)					
		RM≥2 (<i>n</i> =421)	RM≥3 (<i>n</i> =251)	IF≥2 (<i>n</i> =119)	IF≥3 (<i>n</i> =54)	RM + IF≥2 (<i>n</i> =55)	RM + IF≥3 (<i>n</i> =40)
Normal							
5G/5G	72	128	75	38	18	18	13
<i>n</i> (%)	(72 %)	(30.4 %)	(29.9 %)	(31.9 %)	(33.3 %)	(26.6 %)	(32.5 %)
Heterozygote							
4G/5G	27	208	122	55	25	26	17
<i>n</i> (%)	(27 %)	(49.4 %)	(48.6 %)	(46.3 %)	(46.3 %)	(48.8 %)	(42.5 %)
Homozygote							
4G/4G	1	85	54	26	11	11	10
<i>n</i> (%)	(1 %)	(20.2 %)	(21.5 %)	(21.8 %)	(20.4 %)	(25.6 %)	(25 %)
<i>P</i> -value ^a		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

^a Evaluated by Mann-Whitney *U*-test

≥2, IF ≥2, RM + IF ≥2, RM ≥3, IF ≥3 and RM + IF ≥3 subgroups, respectively (Table 2).

Discussion

Two groups of data have been reported on the role of PAI-1 in RM. In the first group, no significant association between the effect of PAI-1 4G gene polymorphism and the risk of pregnancy loss in RM patients was detected [23]. Butchholz et al. (2003) reported a tendency towards the presence of higher frequencies of 4G/4G carriers among patients with a history of two or more consecutive unexplained spontaneous miscarriages (39.1 %) compared to controls (32.3 %), but the difference was not significant (*P*=0.22) [1]. In addition, no association between this polymorphism and early or late pregnancy loss was observed by Gumpel et al. [24].

Furthermore, in a survey by Goodman et al., when RM patients were compared with control women, no differences were detected in the frequencies of heterozygous and homozygous PAI-1 mutations [25].

In contrast, the second group of studies defined an important role for PAI-1 polymorphisms in RM. For instance, Sun et al. confirmed that in women with a history of one or more unexplained miscarriages, homozygosity for PAI-1 4G polymorphism was found significantly more often in RM patients (*p*<0.05) resulting in increased PAI-1 concentrations and hypo fibrinolysis. Therefore, PAI-1 was considered to have a contribution in early pregnancy loss [26]. The association between PAI-1 and RM in Iranian population has been surveyed by Aarabi et al. who reported that patients with homozygote 4G mutations were significantly more prone to RM compared with normal controls [27]. In addition, while PAI-1 heterozygosity for the 4G polymorphism in combination

Table 2 Odds Ratios (OR) and 95 % confidence interval for classifications of case subgroups

		OR	95.0 % confidence interval for Odds Ratios	
			Lower	Upper
RM $\geq$ 2 (<i>n</i> =421)	Heterozygote	4.33	2.64	7.10
	Homozygote	47.81	6.52	350.65
RM $\geq$ 3 (<i>n</i> =251)	Heterozygote	4.34	2.56	7.35
	Homozygote	51.84	6.99	384.69
IF $\geq$ 2 (<i>n</i> =119)	Heterozygote	3.86	2.11	7.07
	Homozygote	49.26	6.43	377.20
IF $\geq$ 3 (<i>n</i> =54)	Heterozygote	3.70	1.749	7.84
	Homozygote	44.00	5.33	363.38
RM + IF $\geq$ 2 (<i>n</i> =55)	Heterozygote	3.85	1.826	8.123
	Homozygote	44.00	5.33	363.38
RM + IF $\geq$ 3 (<i>n</i> =40)	Heterozygote	3.49	1.49	8.13
	Homozygote	55.38	6.52	470.17

with prothrombin G20210A polymorphism heterozygosity was associated with a six fold increased risk of venous thrombosis, homozygosity for the PAI-1 4G polymorphism in combination with G20210A heterozygosity was associated with a 13-fold increased risk [28]. Moreover, our previous study on 100 RM women showed that homozygosity but not heterozygosity for PAI-1 gene polymorphism was significantly higher in women with at least two successive pregnancy losses than the control group [22]. The present study with a larger sample volume, confirmed our previous results and extended them to all six RM and IF subgroups.

Furthermore, using univariate regression analysis, significant differences of PAI-1 genotypes between each of the six case subgroups and the controls showed that the PAI-1 mutation could be considered as a risk factor in all subgroups of patients in both homozygote (4G/4G) and heterozygote (4G/5G) genotypes.

In conclusion, our results suggest that analysis of PAI-1 4G polymorphism may be a helpful strategy in screening of women with RM and IF, although a complementary study on infertile population who do not have RM or IF should be undertaken to further clarify this strategy.

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