

Detection of plasminogen activator inhibitor-1 (-675 4G/5G) gene polymorphism in women with recurrent abortion

Abstract

Background: recurrent abortion is considered one of the most common complications that occur during pregnancy and counts for 15% of pregnancies that are recognized clinically. Many causes can be attributed to the recurrent pregnancy loss e.g. chromosomal anomalies, thrombophilic disorders, uterine anomalies, endocrine abnormalities and fetal anomalies. Thrombophilia can be either hereditary or acquired. Multiple genes had been implicated in the pathogenesis of the thrombophilia. Previous studies have indicated that genetic polymorphism of the plasminogen activator inhibitor-1 gene (PAI-1) may be associated with recurrent abortion.

Aim: The aim of the present study was to investigate whether plasminogen activator inhibitor-1 (-675 4G/5G) gene polymorphism is associated with the occurrence of recurrent pregnancy loss or not.

Methods: DNA samples were collected from sixty six female patients with recurrent abortion (33 primary abortion, 33 secondary abortion) and thirty four healthy controls with normal pregnancy for detection of plasminogen activator inhibitor-1 (-675 4G/5G) gene polymorphism by restriction fragment length polymorphism PCR.

Results: there was a significant association between PAI-1(-675 4G/5G) polymorphism and the occurrence of recurrent pregnancy loss.

Conclusion: Our results assumed that PAI-1 (-675 4G/5G) polymorphism is associated with recurrent pregnancy loss.

Keywords: recurrent abortion, polymorphism, PAI-1, pregnancy, plasminogen, fibrinolysis, pathology, hereditary thrombophilia

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Abbreviations: RPL, Recurrent pregnancy loss; SNP, single nucleotide polymorphism; PAI-1, plasminogen activator inhibitor-1; MTHFR, methylene tetra hydro folate reductase; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism

Introduction

Recurrent pregnancy loss (RPL) or abortion is defined as two times or more fetal loss before the fetus reaches viability. Therefore it includes the duration from the time of conception until pregnancy loss within 20weeks of gestation with an upper limit 24weeks.¹ It can be classified into primary (recurrent pregnancy loss without any previous successful viable pregnancy) or secondary (presence of one or more previous viable pregnancy).² Abortion is considered one of the most common pregnancy related complications that counts for 15% of pregnancies that are recognized clinically.³

Many causes were reported to be associated with the recurrent miscarriage e.g. parental chromosomal anomalies, maternal thrombophilic disorders, structural or functional uterine anomalies, maternal infections and endocrine abnormalities as well as fetal anomalies. However, in the majority of cases no cause is found.⁴ Thrombophilia is defined as an abnormality in blood hemostasis that predispose to thromboembolic events. It can be hereditary or acquired. Importantly, thrombophilia should be differentiated from the hypercoagulable state which is a prothrombotic state and can be physiological or pathological like pregnancy itself.^{5,6} There are many causes of hereditary thrombophilia that are associated with pregnancy

loss including factor V Leiden, prothrombin G20210A gene mutation as well as protein C, protein S and anti-thrombin III deficiency.⁷ The SERPIN plasminogen activator inhibitor-1 (PAI-1) is considered one of the most important inhibitors of fibrinolysis. It acts by inhibition of the plasminogen activators (tissue plasminogen and urokinase plasminogen activators) and blocking conversion of plasminogen into plasmin which is the key step in fibrinolysis inhibition.⁸

PAI-1 gene 4G/5G single nucleotide polymorphism (SNP) include the insertion/deletion of a guanine base, occurs in the PAI-1 promoter region at the 675th position.^{9,10} Both the 4G and 5G alleles contain a binding site for transcriptional activator. On the other hand, the 5G allele possesses an additional binding site for a transcriptional repressor, leading to attenuated transcription and hence reduced PAI-1 formation. Thus, Homozygosity for the deletion genotype (4G/4G) and hetero-zygosity (4G/5G) are associated with higher PAI-1 concentrations than those associated with the insertion genotype (5G/5G), and subsequently reduced fibrinolytic activity.^{10,11} The present study aimed at investigating whether PAI-1 (-675 4G/5G) gene polymorphism is associated with recurrent abortion or not in a cohort of Egyptian women.

Subjects and methods

The present study was conducted on Sixty six female patients admitted to El Shatby University Hospital (Alexandria University, Alexandria, Egypt) with at least two abortions before 20th week of gestation. They were categorized into two groups: group I:33 patients

with primary abortion, group II:33 patients with secondary abortion. Thirty four pregnant females with at least two previous normal uncomplicated pregnancies were recruited as a control group. The exclusion criteria included anatomical uterine disorders, endocrinal dysfunctions, anti-phospholipid syndrome, infectious disorders as well as hereditary thrombophilic disorders including factor V Leiden, methylene-tetra-hydro-folate-reductase (MTHFR) and prothrombin gene GA20210A mutations. Written informed Consent was obtained from all study participants. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Alexandria. All study participants were subjected to full history taking (personal, family history), clinical examination, ultrasound and laboratory investigations for assessment and exclusion of other causes.

Sample collection and DNA extraction

Peripheral blood samples were obtained by venipuncture using a sterile aseptic technique. About 2milliliters of venous blood were withdrawn into EDTA vacutainer tubes. Total genomic DNA was purified according to the manufacturer protocol using the QIAamp DNA Blood Mini Kit, (QIAGEN, and Germany). The quantity and purity of DNA were assayed using a Nanodrop 2000 spectrophotometer

(Thermo Scientific, USA). A260:A230 ratio greater than 1.6 and A260:280 ratios greater than 1.8 were considered indicators for highly pure DNA.

Genotyping

PAI-1 SNP genotyping was carried out by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) technique. Two PAI-1(-675 4G/5G) sequence specific primers were used for amplification of the targeted fragment of PAI-1 gene (Table 1). The PCR reaction was carried out on the SimpliAmp Thermal Cycler (Applied Biosystems, USA). The thermal profile included an initial de-naturation at 95°C for 3minutes, followed by35 cycles of denaturing at 95°C for 30seconds, annealing PAI-1 primers at 58°C for 30seconds and extension at 72°C for 1minute followed by final extension at 72°C for 10minutes. Then, the PCR products were detected by gel electrophoresis on 2% ethidium bromide-stained agarose (Table 1). The BseRI restriction enzyme (Thermo Fisher, USA) was used for post PCR restriction according to the manufacturer instructions. The restriction bands were analyzed on 2% agarose gel electrophoresis.

Table 1 Primers sequence and restriction enzyme used for PAI-1(-6754G/5G) genotyping

Polymorphism	PCR product (bp)	Restriction enzyme	RFLP product (bp)	Primer sequences
PAI-1(-675 4G/5G)	148	BseRI	148a	F : 5'- CCAGAGAGAGTCTGGACACGTGA-3'
			110 & 38b	R: 5'-TGCAGCCAGCCACGTGATTGTCTA-3'

- a. RFLP product for normal allele
- b. RFLP product for mutant allele

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. Comparison between the different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was performed using Fisher’s exact test or Monte Carlo correction. The normally distributed quantitative variables were tested by Student t-test between two groups or by F-test (ANOVA) if more than two groups. For abnormally distributed data comparison was done using Mann Whitney test. Statistical significance was considered at $p<0.05$. The population of the studied sample was explored to find its equilibrium with Hardy-Weinberg equation.

Results

Table 2 describes the distribution of PAI-1 4G/5G SNP in both groups. Forty one patients (62.1%) were of the wild genotype 5G/5G, twenty five patients (37.9%) were of heterozygous genotype 4G/5G. On the other hand, in control group twenty eight females (82.4%) were of the wild type and six females (17.6%) were of the heterozygous genotype. No homozygous genotypic pattern (4G/4G) was detected in both groups. There was a significant difference between both groups regarding the PAI-1 4G/5G SNP genotypic distribution as the 4G allele was more predominant in the patients group compared to the control group ($p=0.038$) suggesting an association between the recurrent pregnancy loss and PAI-1 4G/5G polymorphism.

On further studying of association of the PAI-1 4G/5G SNP with different patient’s characteristics, no significant association was found

between the gene polymorphism (PAI-1 4G/5G) and these variables (Table 3).

Table 2 Comparison between the two studied groups according to PAI-1 4G/5G SNP

	Cases (n=66)		Control (n=34)		P	OR	95% C.I
	No.	%	No.	%			
PAI							
5G/5G®	41	62.1	28	82.4		1	-
5G/4G	25	37.9	6	17.6	0.038*	2.846	1.034 – 7.831
4G/4G	0	0	0	0	-	-	-
Allele frequency							
5G®	107	81.1	62	91.2		1	-
4G	25	18.9	6	8.8	0.061	2.414	0.939 – 6.208

Table 3 Relations between PAI-1 4G/5G SNP and other patients characteristics

Variable		PAI-1 4G/5G SNP						P value
		5G/5G®		5G/4G		4G/4G		
		No	%	No	%	No	%	
Type of abortion	Primary(n=33)	18	54.5	15	45.5	0	0	0.205
	Secondary (n=33)	23	69.7	10	30.3	0	0	
Presence of family history	Yes (n=6)	5	83.3	1	16.7	0	0	0.396
	No (n=60)	36	60	24	40	0	0	
Number of abortions	< 3 (n=26)	15	57.7	11	42.3	0	0	0.55
	≥ 3 (n=40)	26	65	14	35	0	0	

Discussion

Pregnancy is associated with a complex of physiological changes and many complications that can occur to the women. Recurrent abortion is considered one of the most common pregnancy related complications; its frequency is estimated to be from 10-25% of clinically recognized pregnancies. It is a multi-factorial disorder, many risk factors and causes are incorporated into the disease. Yet, the most common cause of pregnancy loss is unexplained.¹² The coagulation pathway has a major role in the placental development and the balance between the clotting and fibrinolytic system is mandatory for a successful implantation.¹³ PAI-1 is considered an important regulator in the fibrinolytic system. So, any abnormality in this gene may affect the normal hemostasis. PAI-1 4G/5G SNP is associated with increased fibrin formation which may affect the placenta circulation and implantation causing pregnancy loss.⁴

In our study, the PAI-1 4G allele of the (-675 4G/5G) SNP showed significantly higher prevalence in females patients with recurrent abortion compared to controls. Our result supports the presence of an association between 4G allele and the occurrence of recurrent pregnancy loss. Our results were consistent with those reported by Elmahgoub and colleagues,¹⁴ who carried out a study on a cohort of Egyptian females studying two SNPs PAI-1 4G/5G and FXIII Val34Leu. They reported a significant higher prevalence of the 4G allele of PAI-1 in cases than controls. Moreover, they assumed that the combination between the two polymorphisms increase the risk of RPL more than the single one.

Similarly, Subrt and his colleagues⁹ stated that the 4G allele of PAI-1-675 4G/5G SNP have an increased risk in RPL in the Czech Republic population. In addition, Magdoud and his colleagues¹⁵ stated the contribution of PAI-1 4G/5G into recurrent pregnancy loss in an ethnically matched cohort of Tunisian females. Furthermore, Li X and his colleagues¹⁶ had conducted a systematic review and meta-analysis for 18 studies conducted on European and worldwide populations that described the effect of PAI-1 polymorphisms on RPL. They stated that the PAI-1-675 4G/5G polymorphism has a potential role in RPL. Moreover, Kamali and colleagues¹⁷ had another meta-analysis for 37 case-control studies for the PAI-1-675 4G/5G SNP and documented increased risk of RPL in females of the Iranian population with PAI-1 4G allele.

On the contrary, Jeddi-Tehrani and colleagues documented that the homozygous genotype only has a significant association with RPL while heterozygosity is not associated with increased risk of RPL.⁴

In our study there was no patients with 4G homozygous genotype in both cases and control groups and this may be explained by the small sample size.

Wolf and his colleagues,¹⁸ stated that they could not assume the relation between the PAI-1 4G mutant allele and RPL in Iranian females. Similarly, Gumpel and his colleagues,¹⁹ proved that this SNP has no role in abortion in Iranian females. They reports that it is only associated with vascular placental insufficiency. In European females, Buchholz and his colleagues,²⁰ found that the combination of homozygosity of both PAI-1 4G/5G and ACE D/I increase the risk of pregnancy loss, while there was no significance difference between cases and control concerning the PAI-1 alone. Our study had some limitations include small sample size and lack of the PAI-1 enzyme assay to correlate the genotype with the phenotype of the patients. Future functional studies on larger Egyptian cohorts are recommended.

Conclusion

From the previously mentioned results, we can conclude that the investigation of PAI-1,-675 4G/5G SNP could be used as a routine investigation in women with recurrent pregnancy loss who could receive personalized prophylactic management.

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None

Conflicts of interest

The author declares that there are no conflicts of interest.

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