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Research Article

Beta-fibrinogen (-455 G/A) and Integrin beta-3 (PLA1/A2) polymorphisms and recurrent pregnancy loss in Gaza strip-Palestine

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ABSTRACT

Background: This study was conducted in order to determine the relationship between the common polymorphisms in *β-fibrinogen* -455 G/A (rs1800790) and *Integrin beta-3* T196C (*PLA1/A2*, rs5918) and recurrent pregnancy loss (RPL) in a group of Palestinian women residing in Gaza strip.

Methods: The study presented here is a retrospective association study with a case-control design. The study population consisted of 102 women who suffered from RPL and 97 healthy women matched for age and without a previous history of RPL. DNA extracted from blood samples of all subjects was investigated for the *β-fibrinogen* (-455 G>A) and *Integrin beta-3* (*PLA1/A2*) polymorphisms using PCR-RFLP and PCR-SSP, respectively.

Results: The study revealed that *β-fibrinogen* -455G/A minor allele frequency (allele A) and its homozygous genotype (AA) were not significantly different between the RPL and the control groups. Likewise, there was no significant difference in the distribution of the *ITGB-3* variant allele (*PLA2*) or *PLA2/A2* genotype between the two groups. *β-fibrinogen* AA genotype was evident in 3.9 % of the patients and in 4.1% of the controls (P-value = 0.94). The frequency of *PLA2/A2* was observed in 2.0 % of the patients and in 2.1% of the controls (P-value = 0.96).

Conclusion: Results of the present study indicate that there is no significant association between the *β-fibrinogen* (455 G>A) or *Integrin beta-3* (*PLA1/A2*) common polymorphisms and the occurrence of RPL in Gaza strip. This non-significant relation indicates that the investigated polymorphisms do not constitute a tangible risk for RPL in our population. Therefore, it is not recommended to consider these two polymorphisms when screening for inherited thrombophilia in Gaza strip.

Keywords: *β-fibrinogen*, *Integrin beta-3*, gene polymorphism, RPL, Gaza Strip, Palestine

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as the occurrence of ≥ 2 consecutive pregnancy losses before 20th week of gestation. This stressful problem is encountered in about 2% of women attempting pregnancy. Known causes of RPL include autoimmune disorders (~20%), endocrine factors (17-20%), anatomic abnormalities (10-15%),

genetic abnormalities (2-5%) and infectious factors (0.5-5%). In an ample number of cases, mounting to 40-50%, however, the causes remain indefinite (Abu-Asab et al., 2011; Lavigne et al., 2005; Ford and Schust, 2009).

In the last two decades, heritable and/or acquired thrombophilias have strongly emerged as a possible etiology for RPL. Thrombophilia can be defined as a

predisposition to form thrombi inappropriately. Marked thrombocytosis, increased platelet aggregation, increased activity levels of coagulation factors, and excess plasma levels of fibrinolytic inhibitors may all enhance thrombosis.

Fibrinogen (Factor I) is a glycoprotein that participates in the coagulation cascade. Fibrinogen is composed of three pairs of polypeptide chains (named α , β and γ) linked by disulfide bonds. Mutations in β -fibrinogen gene lead to thrombotic tendency. The fibrinogen-raising *beta-fibrinogen* gene -455G/A polymorphism may enhance the physiological increase in fibrinogen levels during pregnancy and thereby may predispose to obstetric complications (Leander et al., 2002).

Human platelets carry membrane glycoproteins that control platelet aggregation and activation. A number of clinical studies have suggested that certain polymorphisms of genes encoding these proteins increase the risk for thrombosis (Chiras et al., 2009). Human platelet antigen-1 "HPA-1", GpIIIa or Integrin Beta-3 is a platelet membrane glycoprotein encoded by *ITGB3* gene. The common polymorphism T196C in this gene results in a L59P substitution and is described as the *PLA1/A2* alloantigen system (Chiras et al., 2009; Tan et al., 2012).

Studies on prevalence of *beta-fibrinogen* -455G/A polymorphism among patients with RPL in different populations have yielded conflicting results, whereas some investigators showed an association between this polymorphism and RPL (Goodman et al., 2006; Ticconi et al., 2011; Jeddi-Tehrani et al., 2011; Torabi et al., 2012) others, however, reported the lack of a meaningful association (e.g., Pihusch et al., 2001; Coulam et al., 2006; Yenicesu et al., 2010; Ozdemir et al., 2011)

The trend is not different for *ITGB3 PLA1/A2* polymorphism as some authors stated that this polymorphism constitute a risk factor for RPL (Ruzzi et al., 2005; Ivanov et al., 2010; Lambrinouadaki et al., 2010; Yenicesu et al., 2010; Goncharova et al., 2013), other studies did not confirm such a relation (Pihusch et al., 2001; Ozdemir et al., 2011).

This study was performed in order to investigate the relation between RPL and the common polymorphisms in β -fibrinogen (-455 G>A; "rs1800790") and Integrin beta-3 a/b (*PLA1/A2*; "rs5918") genes among Palestinian RPL women residing in Gaza Strip.

METHODS

Study population

The study was conducted on 102 Palestinian women, 18–35 years old, from Gaza strip who had at least two RPLs ≤ 20 weeks of gestation. Age and ethnicity matched 97 women with at least two live births and without a previous history of abortion or pregnancy-associated complications served as the control group. Informed

consent was obtained from all participants, and approval for conducting the study was obtained from the local ethics committee.

DNA extraction and polymorphism determination

Genomic DNA was extracted from EDTA-blood samples using Wizard Genomic DNA purification Kit (Promega, USA) following the manufacturer's instructions. Genotyping was performed using polymerase chain reaction (PCR) and restriction length fragment polymorphism (RLFP) for β -fibrinogen - 455G>A and sequence-specific (PCR-SSP) for *ITGB3 PLA1/A2*.

The PCR primers (Table I), restriction enzyme and PCR conditions employed in genotyping β -fibrinogen - 455G>A were carried out as described by Xiang-feng et al. (2008). The polymorphism was defined by digesting the PCR product with the restriction endonuclease *HaeIII* (New England BioLabs) at 37°C for 16 hrs. The polymorphic A allele PCR product remains uncut (336-bp) whereas, the wild type G allele is cut into two fragments of 215 and 121-bp.

The *ITGB3 PLA1/A2* polymorphism was determined by PCR-SSP as described by Chiras et al. (2009) using the primers shown in Table 2.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) equation was used to calculate the expected genotypes frequencies. Difference between expected and observed genotypes was assessed by Chi square (X^2) test. *P-values* less than 0.05 were considered statistically significant. The frequencies of the alleles and genotypes were compared between patient and control groups by X^2 test. The odds ratio (OR) and 95% confidence interval (CI) were also estimated in order to test the relation between RPL and the investigated polymorphisms.

RESULTS

-455G>A fibrinogen gene polymorphism and RPL

Genotype and allele frequencies of -455G>A *fibrinogen* gene polymorphism were not significantly different between RPL patients and controls (Table 3). Moreover, statistical analyses of the genotypes under both recessive and dominant models (data no shown) revealed no significant difference between the two study groups.

PLA1/A2 ITGB3 gene polymorphism and RPL

Genotype and allele frequencies of *PLA1/A2 ITGB3* gene polymorphism were not significantly different between RPL patients and controls (Table 4). Additionally, statistical analyses of the genotypes under both recessive and dominant models (data not shown) indicated no significant difference between RPL and control groups.

Table 1: Primer sequences and restriction enzyme for -455G>A fibrinogen.

Primer sequences	PCR products	Restriction enzyme	Digested products
F: 5'-AGGGTCTTTCTGATGTGT-3' R: 5'-AAGTTAGGGCACTCCTCA-3'	336 bp	<i>HaeIII</i>	A allele 336 bp G allele 215+121 bp

Table 2: Primers and lengths of PCR products for *ITGB3* *PLA1/A2* polymorphism.

Primer sequence	PCR products size (bp)
Common forward 5' GGAGGTAGAGAGTCGCCATAG 3'	
<i>PLA1</i> specific reverse primer 5' TCACAGCGAGGTGAGGCCA 3'	90
<i>PLA2</i> specific reverse primer 5' TCACAGCGAGGTGAGGCCG 3'	90

Table 3: Frequency of the -455G>A *fibrinogen* gene polymorphism among RPL patients and control subjects.

Polymorphism	Genotype/Allele	RPL (N=102)	Control (N=97)	Odds Ratio (95% CI)	P-value
<i>fibrinogen</i> -455G>A	GG	70 (68.6%)	62 (63.9%)	1.23 (0.69-2.22)	0.48
	GA	28 (27.5%)	31 (32.0%)	0.81 (0.44-1.48)	0.49
	AA	4 (3.9%)	4 (4.1%)	0.95 (0.23-3.91)	0.94
	G allele	168 (82.4%)	155 (79.9%)	0.85 (0.52-1.41)	0.53
	A allele	36 (17.6%)	39 (20.1%)		

Table 4: Frequency of the *PLA1/A2* *ITGB3* gene polymorphism among RPL patients and control subjects.

Polymorphism	Genotype/Allele	RPL (N=102)	Control (N=97)	Odds Ratio (95% CI)	P-value
<i>ITGB3</i> <i>PLA1/A2</i>	A1/A1	70 (68.6%)	62 (63.9%)	1.23 (0.68-2.22)	0.48
	A1/A2	30 (29.4%)	33 (34.0%)	0.81 (0.44-1.47)	0.48
	A2/A2	2 (2.0%)	2 (2.1%)	0.95 (0.13-6.88)	0.96
	A1 allele	170 (83.3%)	157 (81%)	1.18 (0.71-1.97)	0.53
	A2 allele	34 (16.6%)	37 (19%)		

Hardy-Weinberg equilibrium

Analysis of the observed and the calculated expected genotype frequencies of β -fibrinogen 455G>A and *ITGB3* *PLA1/A2* polymorphisms in the control group showed that the distribution of both genes' genotypes are in Hardy-Weinberg equilibrium.

DISCUSSION

Normal pregnancy is inherently a hypercoagulable state where it is associated with an increase in various physiologic coagulants and a decrease in natural anticoagulants (Brenner et al., 2006; Cernecab et al.,

1997). Genetic variation in any of the several loci involved in the coagulation/fibrinolysis system may further exacerbate this hypercoagulability and result in intervillous or spiral-artery thrombosis, inadequate placental perfusion and could lead to miscarriage (Ford and Schust, 2009). This theory is solidified by the fact that many thrombophilia-associated RPL cases have been successfully treated with administering appropriate anticoagulants (Gris et al., 2004; Brenner et al., 2005).

β -fibrinogen and *ITGB3* proteins are components of the coagulation system and common polymorphisms in the genes (-455G/A and *PLA1/A2*, respectively) encoding those proteins have been linked with thrombophilia and

increased risk of RPL (Goodman et al., 2006; Ticconi et al., 2011; Jeddi-Tehrani et al., 2011; Torabi et al., 2012; Ruzzi et al., 2005; Ivanov et al., 2010; Lambrinouadaki et al., 2010; Yenicesu et al., 2010; Goncharova et al., 2013). Our findings, however, did not replicate this association and revealed that these two polymorphisms do not represent a risk factor for RPL in our population. Meanwhile, results of the present study are in agreement with other reports that also showed lack of association between RPL and -455G/A and *PLA1/A2* (Pihusch et al., 2001; Coulam et al., 2006; Yenicesu et al., 2010; Ozdemir et al., 2011).

Discrepancy between results of association studies conducted on various populations could be due to many reasons including study sample characteristics, population genetic variation(s) unrelated to investigated polymorphism(s), presence of nucleotide polymorphism somewhere else in, or in the vicinity of, the investigated loci, epigenetic alterations and linkage disequilibrium to other sequence variants.

Some authors, due to the multifactorial nature of at least 40-50% the RPL cases, proposed that thrombophilia risk alleles should not be tested individually but as part of a panel of multiple gene variants (Coulam et al., 2006; Goodman et al., 2006; Torabi et al., 2012;). Despite our agreement on that proposition we still believe that each allele in this panel, when tested alone, should show significant difference between RPL patients and control subjects. Furthermore, no international consensus will be attained on the component polymorphisms of such a panel and perhaps each population will have its own particular panel.

In light of the obtained results we do not recommend inclusion of β -fibrinogen -455G/A and *ITGB3 PLA1/A2* polymorphisms when screening idiopathic RPL cases for inherited thrombophilia in our population.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Local Ethics Committee

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