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The Role of Vascular Endothelial Growth Factor Gene Polymorphisms in Recurrent Spontaneous Abortions in Saudi Women

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Abstract

Vascular endothelial growth factor (VEGF) genes regulate proliferation of vascular endothelial cells, hence, are essential for both physiological and pathological angiogenesis. The aim of the present study was to examine the association between three common VEGF single nucleotide polymorphisms (-583 T/C, -1154 G/A, and 936 C/T) and the risk of RSA in Saudi women. A total of 200 women; 100 with RSA-patient matched with 100 controls were enrolled sequentially. Genotyping was conducted using TaqMan assay in order to detect single nucleotide polymorphism (SNPs) in DNA extracts from blood samples. Results showed that the +936C/T allele of VEGF gene is not associated with increased risk of RSA. On the other hand, polymorphisms within -583T/C and -1154G/A of the promotor region were significantly associated with increased RSA risk. Based on these it can be concluded that polymorphisms of VEGF are associated with increased risk of RSA in Saudi women. Hence VEGF gene polymorphism can be a useful biomarker to predict susceptibility to RSA.

Keywords: Genotyping; Pregnancy Loss VEGF; Recurrent of Spontaneous Abortion; -583T/C; -1154G/A

Introduction

Recurrence of spontaneous abortion (RSA) is a frequent reproductive complication that involves 3 consecutive spontaneous abortions [1]. Numerous etiologic causes including; chromosomal abnormalities, antiphospholipid syndrome, structural uterine abnormalities, endocrine disorders, immunologic factors, bacterial and viral infections, and environmental factors; have been documented as leading causes for RSA [2]. However, the exact underlying molecular vascular pathophysiologic mechanism has been poorly understood.

Genetic variability has been suggested to play a role in RSA, and over one hundred proposed genes were investigated [3]. In addition, there is strong evidence supporting a close relationship between embryonic development and the state of vascularization of the chorionic villi [4,5]. Vascular endothelial growth factor (VEGF) is a well-known key regulator in angiogenesis [6]. The VEGF protein family includes VEGF-A (VEGF), VEGF-B, VEGF-C, VEGF-D, placenta growth factor (PGF), and their VEGF receptor family (VEGFR-1/ Flt-1, VEGFR-2/KDR, and VEGFR-3/Flt-4) [7,8].

The VEGF gene is highly polymorphic, and at least 25 different polymorphisms have been identified [9]. Some of these polymorphisms are functional and found to be correlated with variations in the production of VEGF protein [10-13]. Furthermore several studied suggested a diminished immunoreactivity of placental trophoblastic vascular endothelial growth factor (VEGF), in the deciduae endothelium, is associated with spontaneous miscarriages [14]. Therefore, polymorphisms in the VGEF are possibly associated with risk of RSA.

In view of the review summarized above, the aim of the present study was to evaluate the association between three common VEGF single nucleotide polymorphisms, -583 T/C and -1154 G/A, in the promoter region, and 936 C/T in the 3'-untranslated region, and the frequency of occurrence of RSA in Saudi women.

Materials and Methods

Participants

Participants were 200 women age (18-45 years), with un-explained RSA, consecutively referred to King Khaled University Hospital abortion clinic, Saudi Arabia. All Participants were attending the clinic for routine check-up. The protocol of the present study was approval by the IRB at King Khalid University Hospital and Ethical Committee of King Saud University. All participants signed an informed consent with their approval to participate in the study.

Study design

A prospective case-controlled design was conducted using two female groups. Patients with unexplained reoccurrence of spontaneous abortion (RSA) were identified sequentially as cases group, hence designated as RSA-group. A second group, matched with age and BMI, served as control group. The inclusion and exclusion criteria for both groups were outlined as follows:

RSA-Group: A hundred women age (18-45) years and BMI (25-30) with unexplained RSA attending the outpatient clinics for abortion, at the Department of Obstetrics and Gyneacology at the King Khalid University Hospital, Riyadh or other hospitals, consecutively referred. The inclusion criterion is; minimum of three RSA. Women with RSA in whom the causes of abortion were known, were excluded by performing anatomical, hormonal, and chromosomal tests. Infection tests for toxoplasma, cytomegalovirus, rubella virus, hepatitis B and C viruses, HIV carried out on these females. In addition women with

RSA with autoimmune causes, including anti-cardiolipin antibody also excluded from the study.

Control Group: The control group consisted of 100 healthy Saudi females age (18-45) and BMI (25-30), with no history of abortion, with at least two successful pregnancies who were recruited from King Khalid University Hospital.

DNA Quantification and Extraction

Blood samples (8 ml) for DNA were withdrawn by venepuncture from both groups, in tubes containing anti-coagulant EDTA. The blood centrifuged at 3500 rpm for ten minutes, to separate the plasma from the cells and the buffy coat. The plasma, buffy coat and cells were carefully separated and the plasma stored at -80°C until required for analysis. DNA was extracted from whole blood using pure gene DNA purification kit. The concentration and purity of each DNA sample was determined using the Nano Drop 2000 Spectrophotometer. From each sample 100 µl working DNA (50 mg/µl) will be labelled and stored at 4°C or -20°C.

Genotyping Assays

TaqMan assay was used to detect SNPs in DNA extracted from samples. Specific primers and probes for the TaqMan genotyping method were available from Applied Bio systems for both SNPs and were used according to manufacturer's instructions. Briefly, this protocol provides instructions for real-time reverse transcription-PCR (real-time RT-PCR) using TaqMan Gene Expression Assays and TaqMan Non-coding RNA Assays. Each assay has a specific probe labelled with a unique fluorescent dye, resulting in different observed colors for each assay [15].

Statistical analysis:

Descriptive measures and correlation matrix were generated to describe the bivariate linearity among variables of interests by Pearson Product moment correlation (r). Chi square test was carried out to determine the difference in frequencies of the patient and control groups. The odd ratio's was calculated to examine the association between genotype and the phenotype characteristics. P value of (0.05) was adopted as the level of significant. All statistical analysis was conducted using SPSS program version 20.

Results

The results of Chi square test showed that there were no significant (p>0.05) association between the +936C/T (rs3025039) allele of VEGF gene and increased risk of RSA (Table 1). On the other hand, two VEGF promotor region alleles (-583T/C (rs3025020) and -1154G/A (rs1570360) were significantly (p<0.05) associated with increased RSA risk (Tables 2 and 3).

VEGF (+936) C/T (SNP NO.: rs3025039)						
Genotype	Control No. (%)	Patients (%)	Control vs. Patients			
			OR	CI	χ2	p-value
CC	74 (74%)	67 (73%)	0.71	0.39 – 1.31	1.178	0.277
CT	20 (20%)	24 (24%)	1.26	0.65 – 2.47	0.466	0.494
TT	6 (6%)	9 (3%)	1.55	0.53 – 4.53	0.649	0.42
Total	**100**	**100**				
Allele	**Control (Freq)**	**Patients (Freq)**	**Control vs. Patients**			
			OR	**CI**	**χ2**	**p-value**
C	0.84	0.79	0.72	0.36 – 1.42	0.914	0.339
T	0.16	0.21	1.4	0.80 – 2.43	1.4	0.236

Table 1. The genotype and allele frequencies of VEGF (+936) C/T

VEGF (-583) T/C (SNP NO.: rs3025020)						
Genotype	Control No. (%)	Patients (%)	Control vs. Patients			
			OR	CI	χ2	p-value
TT	15 (15%)	2 (2%)	0.12	0.03 – 0.52	10.86	0.009
TC	65 (65%)	70 (70%)	1.26	0.69 – 2.27	0.57	0.45
CC	20 (20%)	28 (28%)	1.56	0.81 – 3.00	1.754	0.185
Total	**100**	**100**				
Allele	**Control (Freq)**	**Patients (Freq)**	**Control vs. Patients**			
			OR	**CI**	**χ2**	**p-value**
T	0.475	0.37	0.65	0.40 – 1.04	3.181	0.047
C	0.525	0.63	1.63	0.98 – 2.71	3.575	0.058

Table 2. The genotype and allele frequencies of VEGF (-583) T/C

VEGF (-1154) G/A (SNP NO.: rs1570360)						
Genotype	Control No. (%)	Patients (%)	Control vs. Patients			
			OR	CI	χ2	p-value
GG	70 (70%)	3 (3%)	0.01	0.05 – 0.95	96.84	0.001
GA	21 (21%)	64 (64%)	6.69	3.56 – 12.6	37.831	0.005
AA	9 (9%)	33 (33%)	4.98	2.23 – 11.1	17.36	0.003
Total	**100**	**100**				
Allele	**Control (Freq)**	**Patients (Freq)**	**Control vs. Patients**			
			OR	**CI**	**χ2**	**p-value**
G	0.805	0.35	0.13	0.07 – 0.23	55.74	0.001
A	0.195	0.65	7.67	4.49 – 13.0	61.21	0.005

Table 3. The genotype and allele frequencies of VEGF (-1154) G/A

The results of Chi square test showed that there were no significant (p>0.05) association between the +936C/T (rs3025039) allele of VEGF gene and increased risk of RSA (Table 1). On the other hand, two VEGF promoter region alleles (-583T/C (rs3025020) and -1154G/A (rs1570360) were significantly (p<0.05) associated with increased RSA risk (Tables 2 and 3).

Discussion

The major findings of the present study showed that the two promoters regions -583 T/C and -1154 G/A polymorphisms and allele haplotypes are associated with increased risk of RSA. The gene of VEGF is a member of growth factor family. It encodes a heparin-binding protein, which exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis. Clearly disruption of this gene results in abnormal formation of blood vessel and the subsequent abnormal placental angiogenesis [16,17]. It was reported that VEGA gene is up-regulated in many known tumors and its expression is correlated with tumor stage and progression. Furthermore, allelic variants of VEGA gene have been associated with microvascular complications of diabetes 1 (MVCD1), atherosclerosis, cerebrovascular and cancer diseases [11,18].

On the contrary, the findings of the present study regarding the non-significant association of +936 C/T polymorphism and allele haplotypes of RSA-women, are in agreements with previously reported [19] who found no association between RSA and the +936 C/T VEGF polymorphisms. Similar results had been published [6]. Despite that other VEGF polymorphisms have been found to be associated with RSA [17]. It is important to realize that these studies comprised women with only two miscarriages whereas our study included only women with three or more miscarriages.

The findings regarding significant association between VEGF promoter region genotype and allele frequencies in RSA-patients while genotype TT as well as the allele T frequency were remarkably associated with decreased risk to RSA are consistent with previously reported in Bahraini women [20,21]. Here we extended previous findings by analysing the distribution of 5′-UTR of intron 6 2583C/T VEGF polymorphisms in Saudi RSA-women. Based on these extended works it can be generalized that genotypes -1154 AA and GA are significantly more frequent in women with RSA compared with controls wherein the genotype GG is not associated with RSA. The allele -1154 A, but not G, is associated with increased risk of RSA. Similar findings was reported that showed that the -1154A allele was increased substantially, when -1154 A/A genotype was considered, in Indian RSA-women [22]. In addition, the frequency of homozygosity of the VEGF-1154 G/A gene was significantly higher among women experiencing recurrent implantation failure, compared with fertile control women. It was concluded that homozygosity of the VEGF -1154 G/A gene may be considered a susceptibility factor affecting for recurrent implantation failure [23]. Given recurrent implantation failure shares pathogenic similarities with RSA, VEGF -1154 G/A may play a critical role in RSA. On contrary, Zhang and colleagues reported that there were no association between VEGF -1154 G/A polymorphism and RSA-Asia women, as compared with non-RSA Asian women [24]. However, the results of systematic review and meta-analysis suggested that -1154G/A (rs1570360), +936C/T (rs3025039), and -583T/C (rs3025020) polymorphisms correlated with an elevated risk of RSA, indicating a possible increased risk for RSA with polymorphisms at those 3 sites [25,26].

Conclusion

The findings of the present study confirmed that polymorphisms in VEGF gene is associated with the presence of increased RSA risk. Hence can be useful biomarkers to predict susceptibility to RSA. However, additional parameters such as predictive values, sensitivity, specificity, ROC and logistic regression must be considered in the design of future studies in order to establish cause and effect between VEGF geneses and RSA.

Conflict of Interests

The author has no conflict of interest.

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