RESEARCH ARTICLE

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Potential Impact of Vascular Endothelial Growth Factor Gene Variation (-2578C>A) on Breast Cancer Susceptibility in Saudi Arabia: a Case-Control Study

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Abstract

Aim: VEGF gene polymorphisms can induce either increase or inhibition of VEGF secretion, with altered promoter activity. The VEGF rs699947 SNP is located in the promoter region and is associated with susceptibility to breast carcinoma development. Here, we investigated the association of the -2578C>A polymorphism in the VEGF gene with breast cancer risk in Saudi women. **Methodology:** Genotyping of the VEGF-gene variation (-2578A>C) was performed using the amplification refractory mutation system PCR. We investigated the association of VEGF gene variants with different clinicopathological features of breast cancer patients. **Results:** A significant difference was observed in genotype distribution among the breast cancer cases and sex matched healthy controls (p=0.03). The frequencies of the three genotypes CC, CA, AA found in the patient samples were 37%, 45% and 18% and in the healthy controls were 54%,37% ,and 09% respectively. An increased risk of developing breast cancer in Saudi women was associated with the VEGF −2578 AA genotype (OR = 2.91, 95 % CI, 1.18-7.20; p = 0.01; RR 1.78 (1.01-3.11 p=0.01), the VEGF −2578 A allele (OR = 1.79, 95 % CI, 1.17-2.73; p = 0.004: RR 1.35 1.07-1.71) and the VEGFR-(CA+ AA) (OR 1.99 1.13-3.51; RR 1.401.0-1.85). Thus the A allele increased the risk of BC when compared with C allele. When we stratified groups of patients according to the status of tumor markers, stage, age and metastasis, statistically significant associations with −2578 C/A SNP were revealed. **Conclusion:** Our data showed a significant association of the VEGF -2578C>A polymorphism with BC susceptibility in Saudi women. The VEGF -2578AA homozygote significantly increases the risk and can be useful as a predisposing genetic marker. Further studies with larger sample sizes are necessary to confirm our findings.

Keywords: VEGF-Vascular endothelial growth factor- SNP- Single-nucleotide polymorphism- UTR-Untranslated region

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Introduction

Breast cancer is the most frequently diagnosed noncutaneous malignancy in women worldwide, which affects more than 1 million women annually (Bray, 2013; Babu, 2013). Saudi Arabia has the lowest rate of breast cancer incidence in the Arab world. The nationwide average of incidence in the Kingdom is 22 patients for every 100,000 women. In the UAE, 23 patients, Kuwait 46 patients, Jordan 49 patients, Qatar 48 patients and Bahrain 53 patients, for every 100,000 women Chouchane et al., 2014).There is a substantial rise in the incidence of breast cancer in Saudi Arabia in recent years, particularly among younger females compared to affected females' in western countries (AlJohani et al., 2016). Although the etiology of Breast cancer is entirely unknown, there is abundant evidence that genetic factors play key roles in the pathogenesis and progression of Breast cancer (Hashemi et al., 2013). The human VEGF gene consists of eight exons separated by seven introns that exhibit alternative splicing to form a family of proteins (Vincenti et al.,1996) and plays a key role in a number of pathological processes including angiogenesis, tumor growth, and metastasis. Angiogenesis is a vital step in the development of cancer and is necessary for primary tumor growth, invasiveness, and metastases. Overexpression of VEGF was found in several tumor tissues (Nakamura et al., 2002) . Breast cancer is involving lymph angiogenesis, which is the recruitment of blood and lymphatic vessels, to a growing tumor (Schoppmann et al., 2002). Large number of evidences from in vitro and in vivo experiments has shown that increased VEGF expression is associated with tumor growth and metastasis (Ferrara et al., 2002). Furthermore, the inhibition of VEGF signaling results in suppression of both tumor-induced angiogenesis and tumor growth (Ferrara et al., 2003).Pharmacogenomics studies

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have reported associations between single nucleotide polymorphisms (SNPs) in VEGF and VEGFR-2 and bevacizumab response in metastatic breast cancer Thus, genetic variability in VEGF and VEGFR-2 represents a logical candidate to study as a potential biomarker for bevacizumab (Schneider et al., 2012). Previous data have suggested that single nucleotide polymorphisms within VEGF gene have biologic importance in predicting risk and prognosis of cancer including breast cancer .It has been indicated that gene polymorphism in the promoter reagion, intron, exon, or untranslated regions (3′- and 5′-UTR) may affect the production or function of the corresponding protein (Ruggiero et al., 2011).

Till date some VEGF gene polymorphisms have been reported some of them are depicted in Figure 1. The -2578C/A (rs699947) SNP in its promoter region and the +405G/C (rs2010963) SNP in the 5'-untranslated region of the VEGF gene are associated with altered VEGF secretion (Almawi et el., 2013). Accordingly, these polymorphisms have been suspected to correlate with the progression and prognosis of cancer. Angiogenesis and inflammation are implicated in breast cancer prognosis; however, the role of individual germline variation in related genes is unknown. Studies assessing the effect of antiangiogenesis drug bevacizumab (BEV) on breast cancer (BC) outcome have shown different effects on progression-free and overall survival, suggesting that a subgroup of patients may benefit from this treatment (Hein et al., 2015).

The high levels of circulating VEGF have been observed in individuals with various malignancies including breast (Heer et el., 2001), uterine (Moon et al., 2000), gastrointestinal (Karayiannakis et al., 2002) , lung (Kishiro et el., 2002) and prostate (Li et al., 2005). Several studies investigated the VEGF genetic polymorphisms in Breast cancer in different ethnic groups and led to different conclusions (Rahoui et al; 2014; James et al 2014; Chen et al., 2014; Luo et al., 2013; Rodrigue et al., 2012; Wang et al., 2011; Kapahi et al., 2015) however, the results have been inconsistent, suggesting that the association between the VEGF -2578C/A polymorphism and cancer requires further investigation.

To the best of our knowledge, there are no reports concerning the impact of VEGF -2578C>A gene polymorphism on Breast Cancer risk in Saudi Arabian women. Hence, this study aimed to evaluate the possible association between VEGF -2578C>A polymorphism with risk or protection of Breast Cancer women of Saudi Arabian.

Materials and Methods

The study included clinically, histologically, pathologically and radiologically confirmed cases of Breast cancer .This population-based case–control study was done on 100 cases and 100 gender matched healthy women with no history of any types of cancer and not related to the patients.

The exclusion criteria include Patients unwilling or unable to comply with the protocol. Patients with a history of previous cancer or metastasized cancer from other organs except Breast were excluded. After assessing the clinicopathological findings, a 4ml sample of peripheral blood was collected by venipuncture in EDTA tubes from each patient and healthy control.

DNA extraction

The DNA was extracted by using DNeasy Blood Kit (cat 69506) from Qiagen (Germany) as per the manufactures instructions. The extracted DNA was dissolved in nuclease-free water and stored at 4°C until use. Quality and integrity of DNA were checked by NanoDrop™ (Thermo Scientific, USA).

VEGF -2578C/A (rs699947) genotyping

VEGF -2578C>A genotyping was detected by using amplification-refractory mutation system –PCR (ARMS-PCR). ARM Systems are based on the use of sequence-specific PCR primers that allow amplification of test DNA only when the target allele is contained within the sample. Following an ARMS reaction the presence or absence of a PCR product is diagnostic for the presence or absence of the target allele. The VEGF -2578C>A genotyping primers were designed by using primer3 software as depicted inTable 1.

The ARMS-PCR was performed in a reaction volume of 25uL containing template DNA (50ng), FO -0.30uL , RO -0.30uL , RI -0.20uL , RI -0.20uL of 25pmol of each primers and 10uL from GoTaq® Green Master Mix (cat no M7122) (Promega,USA). The final volume of 25uL was adjusted by adding nuclease free ddH2O . Finally, the 2ul of DNA was added from each patient.

Thermocycling conditions

The amplification conditions used were at 95 °C for 10 minutes followed by 40 cycles of 94oC for 35sec, 58 °C for 40 sec, 72 °C for 45 sec followed by the final extension at 72 °C for 10 minutes.

The amplification products were separated by electrophoresis through 2% agarose gel stained with 0.5μg/mL ethidium bromide and visualized on a UV transilluminator. Primers FO and RO flank the exon of the VEGF -2578C>A gene, resulting a band of 353bp to act as a control for DNA quality and quantity. Primers Fwt and RO amplify a wild-type allele (C allele), generating a band of 229 bp, and primers FO and Rmt generate a band of 149bp from the mutant allele (A allele) as depicted in Figure 2.

The best temperature was determined to be 58°C in the temperature range of 55°C to 63°C tested with a gradient PCR thermocycler. The annealing temperature was lowered from 60 to 58°C to favor the binding of both forward wild and reverse mutant primers that contain mismatches to the templates. The number of cycles was increased from 30 to 40 cycles, significantly enhancing the yields of all three PCR products. Together, these changes resulted in a more robust amplification of the mutant allele and a less competing reaction from the control, as shown by the relative intensities of the corresponding bands on agarose gel electrophoresis.

Statistical analysis

Deviations from Hardy-Weinberg disequilibrium (HWD) was calculated by Chi-square (χ^2) goodness-of-fit test. Group differences were compared using Student's two-sample t-test or one-way analysis of variance (ANOVA) for continuous variables and Chi-squared for categorical variables. Differences in the VEGF gene allele and genotype frequencies between groups were evaluated using Chi-square test. The associations between VEGF -2578C>A genotypes and risk of breast cancer were estimated by computing the odds ratios (ORs), risk ratios (RRs) and risk differences (RDs) with 95 % confidence intervals (CIs). Allele frequencies among cases as well as controls were evaluated by using the Chi–square Hardy-Weinberg equilibrium test. A p-value < 0.05 was considered significant. All statistical analyses were performed using Graph Pad Prism 6.0 and SPSS 16.0.

Results

The Hardy-Weinberg Equilibrium Analysis: The genotype distributions and allele frequencies of the SNPs rs699947 located in the VEGFR gene showed no deviation from HWE (χ^2 = 0.44 P=0.612) in the patient group similarly the genotype distributions and allele frequencies of the SNPs rs699947 showed no deviation from HWE (χ^2 = 0.52 p=0.712) in the control group. Thus, we chose 10% samples from normal control group randomly to review genotyping results, showing that the accuracy rate was more than 99%.

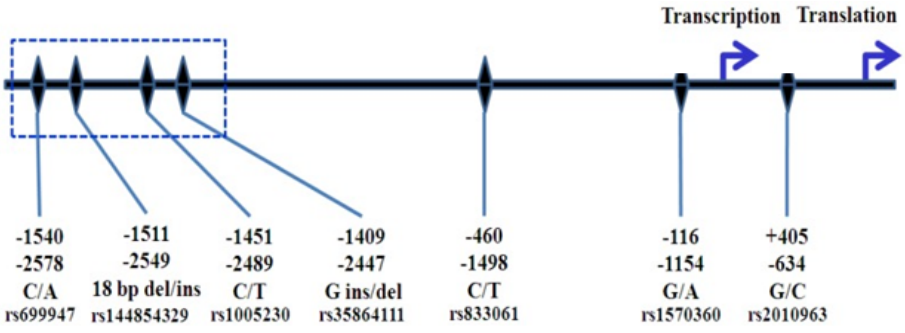
Study population

All demographic features of the subjects are depicted inTable 3. In brief, a total of 100 Breast cancer patients and the same number of gender matched healthy control were analyzed. This research was approved by the Research ethics committee, University of Tabuk and written informed consent was obtained from all the subjects before enrollment. Blood (4ml) samples were collected from participants in EDTA tubes.

Of 100 consecutive breast cancer patients, 32 (32%) patients were below or equal to 40 years age and 68 (68%) were above 40 years of age. Of breast cancer cases 37 (37%) were in early (I and II) stage and 63 (63%) cases were in advanced stages (III and IV). Histological grading of the patients tumor showed that 14 (14%), 33 (33%) and 53 (53%) were in grade I, II and III respectively. Out of 100 cases, how distant metastasis. Metastasis status of patients showed that 65 (65%) patients had distant metastasis and 35 (35%) do not show distant metastasis. Based on the receptor status, out of 100 Breast cancer cases 48 (48%) were positive for Her2/neu, 67 (67%) were carrying estrogen receptor and 64(64%) were +ve for progesterone receptor.

Case-control genotype distribution

This study observed that high percentage of CA (45%) and AA (18%) genotype was found in patients compared to controls CA (37%) and TT (9%) genotype while lower CC (37%) genotype in patients compared to control CC (54%) genotype as depicted in Table 4. We observed a statistically significant difference in the frequencies of VEGF rs699947 genotypes among patients and gender matched healthy controls (P=0.030).The frequency of A allele (fA) was found to be higher among breast cancer patients (0.41) whereas, the lower frequency of A allele (fA) was observed among healthy controls (0.28). However the frequency of C allele (fC) was found to be lower among breast cancer patients (0.59) than the healthy controls (0.72) as depicted inTable 3.



Nomenclature of *VEGFA* SNPs. The positions in the upper row correspond to the transcription start site, while the positions in the lower row correspond to the translation start site. The polymorphisms in the box are in complete LD. - 2578C is linked with - 2549 18 base pairs deletion, - 2489C. and - 2447G insertion. - 2578A is linked with - 2549 18 base pairs insertion, - 2489T and - 2447G deletion.

Figure 1. Nomenclature of *VEGFA* SNPs

Table 1. Amplification- Refractory Mutation System –PCR Primers for VEGF -2578C>A Gene Polymorphism

Direction		Primer Sequence	AT	Product size
FO- VEGF		5-CCTTTTCCTCATAAGGGCCTTAG-3	58°C	353bp
RO- VEGF		5-AGGAAGCAGCTTGGAAAAATTC-3		
FI –VEGF (Fwt)	A allele	5-TAGGCCAGACCCTGGCAA-3		149bp
RI- VEGF (Rmt)	C allele	5-GTCTGATTATCCACCCAGATCG-3		243bp

Fo-outer forward primer, Ro-Reverse outer primer; AT-annealing temperature; FI-Inner forward primer, RI-Inner Reverse primer

Table 2. Preparation of PCR Cocktail for VEGF -2578C>A Polymorphism

	1x
PCR master mix	10ul
Forward primer FO	0.30 ul
Reverse primer RO	0.30 ul
Forward primer FI A	0.20 ul
Reverse primer RI C	0.20 ul
Nuclease free water	12ul
Total volume	23ul
DNA (50ng)	2ul

Correlation between VEGF rs699947C>A gene variation and age

As depicted in Table 4, statistical analysis of the correlation between VEGF rs699947C>A gene variation in breast cancer patients revealed highly significant associations with age status (p=0.042) .The distribution of AA genotype increased significantly among younger patients (<40 years of age) (37.5% vs 8%) whereas the frequency of heterozygosity CA increased significantly among older cases (>40 years of age) (48% Vs 3%) .

Correlation between VEGF gene variation (rs699947C>A) and stage status

As depicted in Table 4, statistical analysis of the correlation between VEGF rs699947C>A gene variation in breast cancer patients revealed highly significant associations with stage status (p=0.042) .The distribution of the CA and AA genotype increased significantly with increasing the breast stage like CA (53% Vs 29.72%) but AA genotype remains almost the same among the different stages (18.91% vs 17.46%).

Table 3. Clinicopathological Characteristics of Breast Cancer Patients

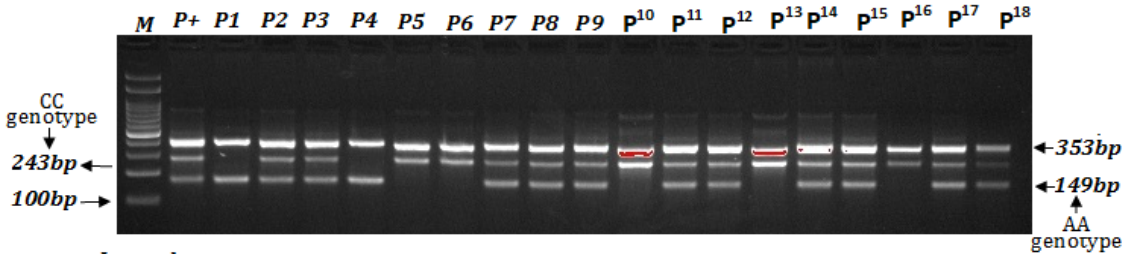
Parameters	No	%	Healthy Controls %
Patients	100	100%	100 (100%)
Age Group			
Age >40	68	68%	
Age<40	32	32%	
Stage			
Early (I & II)	37	37%	
Advanced (III & IV)	63	63%	
Grading			
Grade I	14	14%	
Grade II	33	33%	
Grade III	53	53%	
Estrogen receptor			
Positive	67	67%	
Negative	33	33%	
Progesterone Receptor			
Positive	64	64%	
Negative	36	36%	
Her2/neu			
Positive	48	48%	
Negative	52	52%	
Distant Metastasis			
Positive	65	65%	
Negative	35	35%	

Correlation between VEGF rs699947C>A gene variation and histological grade status

As depicted in Table 4, statistical analysis of the correlation between VEGF rs699947C>A gene variation in breast cancer patients with histological grade status revealed no significant associations in different grades

Table 4. Allelic Frequencies of VEGF-(-2578 C>A) Polymorphism in Cases and Controls

Subjects	N=	CC	AA	CA	A allele	C allele	P-Value
Cases	100	37 (37%)	18 (18%)	45 (45%)	0.41	0.59	p=0.0303
Controls	100	54 (54%)	09 (9%)	37 (37%)	0.28	0.72	
Significance				X^2=6.96 , df:2			



Legend

- M-Marker -100bp
- CC genotype-243bp , AA genotype-149bp : control band -353bp
- P+ positive control (heterozygous)
- P2,P3,P7,P8,P9,P11,P12,P14, P15 .P17.P18 (Heterozygous)-A & C are present
- P1,P4-(Homozygous)-AA genotype
- P5,P6,P10,P13,P16 (Homozygous)-CC genotype

Figure 2. Detection of VEGF-2578C/A (rs699947) Genotyping by ARMS-PCR in Breast Cancer Patients and Healthy Controls

Table 5. Correlation between VEGF (-2578C>A) Polymorphisms and Clinicopathological Characteristics of Breast Cancer (BC) Patients.

Parameters	N=	CC %	CA %	AA %	X^2	
Age Group						
Age >40	68 (68%)	29 (42.60)	33 (48.52)	6 (8.82)	12.36	p=0.002
Age<40	32 (32%)	08 (25)	12 (3.84)	12 (37.5)		
Stage status						
Early (I & II)	37 (37%)	19 (51.35)	11 (29.72)	07 (18.91)	6.34	p=0.042
Advanced (III & IV)	63 (63%)	18 (50)	34 (53.96)	11 (17.46)		
Grading status						
Grade I	14 (14%)	6 (42.85)	5 (35.71)	03 (21.42)	0.66	p=0.94
Grade II	33 (33%)	11 (33.33)	16 (48.48)	06 (18.18)		
Grade I	14 (14%)	6 (42.85)	5 (35.71)	03 (21.42)	0.74	p=0.69
Grade III	53 (53%)	20 (37.73)	24 (45.28)	09 (16.98)		
Estrogen receptor status						
Positive	67 (67%)	26 (38.80)	38 (56.70)	06 (8.95)	12.45	p=0.002
Negative	33 (33%)	11 (33)	10 (30.30)	12 (36.36)		
Progesterone Receptor status						
Positive	64 (64%)	23 (35.93)	30 (46.87)	11 (17.18)	0.61	p=0.80
Negative	36 (36%)	14 (38.88)	15 (41.66)	07 (19.44)		
Her2/neu status						
Positive	48 (50%)	15 (31.25)	27 (56.25)	08 (16.66)	3.35	p=0.18
Negative	52 (52%)	22 (42.30)	18 (34.61)	10 (19.23)		
Distant Metastasis status						
Positive	65 (65%)	22 (33.84)	37 (56.92)	06 (9.23)	14.3	p=0.008
Negative	35 (35%)	15 (42.85)	08 (22.85)	12 (34.28)		

(p=0.094).

Correlation between VEGF rs699947C>A gene variation and receptor status

As depicted in Table 4, statistical analysis of the correlation between VEGF rs699947C>A gene in breast cancer patients with estrogen receptor status revealed highly significant associations (p=0.02) except for PR and her2/neu status revealed non significant associations. VEGF rs699947 polymorphism was associated with ER expression (p=0.02) as manifested by a higher distribution of (CA) genotypes in ER- positive than in ER- negative patients (56% versus 30%).

Correlation between VEGF rs699947C>A gene variation and metastasis status

As depicted in Table 4, statistical analysis of the correlation between VEGF rs699947C>A gene in breast cancer patients with metastasis status of breast cancer patients revealed a strong significant association (p =0.008). The distribution of heterozygosity (CA) was higher in distant metastatic cases (56.92%).

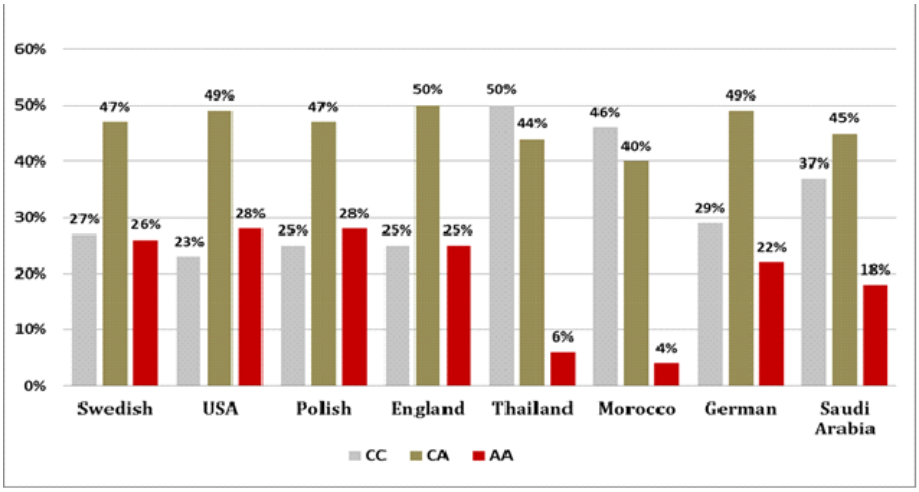


Figure 3. Distribution of VEGFA rs699947C> A Gene Polymorphism in Breast Cancer in the World

Table 6. Association of VEGF rs699947 C>A Gene Variation with Breast Cancer

Genotypes	Healthy controls		Breast cancer patients		OR (95% CI)	Risk Ratio (RR)	P-Value
	(N=100)	%	(N=100)	%			
Codominant							
VEGF-CC	54	54%	37	37%	1 (ref.)	1 (ref.)	
VEGF-CA	37	37%	45	45%	1.77 (0.97-3.24)	1.31 (0.98-1.76)	0.06
VEGF-AA	9	9%	18	18%	2.91 (1.18-7.20)	1.78 (1.01-3.11)	<0.021
Dominant							
VEGF-CC	54	54%)	37	37%	1 (ref.)	1 (ref.)	
VEGF- (CA+ AA)	46	46%	63	63%	1.99 (1.13-3.51)	1.40 (1.0-1.85)	<0.016
Recessive							
VEGF- (CC+ CA)	101	91.80%	100	84.74%	1 (ref.)	1 (ref.)	
VEGF-AA	9	8.20%	18	15.25%	2.02 (0.86-4.7)	1.50 (0.86-2.61)	0.1
Allele							
VEGF-C	155	73.80%	137	31%	1 (ref.)	1 (ref.)	
VEGF-A	55	26.20%	81	69%	1.66 (1.10-2.51)	1.31 (1.04-1.65)	<0.020

Table 7. Distribution of VEGFA rs699947 C>A Polymorphism Genotypes in Healthy Controls

Country	Ethnicity	Controls	CC	AC	AA	Author
UK	European	66	13	38	15	Yang, 2003
Japan	East Asian	203	93	91	19	Awata, 2005
Poland	European	91	29	43	19	Buraczynska,2007
Australia	European	93	26	43	24	Abhary, 2009
Australia	European	181	45	91	45	Abhary, 2009
Japan	East Asian	292	163	107	22	Nakamura, 2009
Korea	East Asian	134	92	36	6	Chun, 2010
China	East Asian	138	82	51	5	Yang, 2011
Saudi Arabia	Middle east	100	54	37	9	Our study

Risk of Breast Cancer with VEGF rs699947 C>A gene polymorphism in BC patients

A multivariate analysis based on logistic regression like odds ratio, risk ratio and risk difference with 95% confidence intervals were calculated for each group to estimate the association between the VEGF rs699947 variant and risk of Breast cancer in Saudi patients. Odds ratio and risk ratio with 95% confidence intervals were calculated for each group to estimate the degree of association between the VEGF rs699947 variant and risk of Breast cancer risk in Saudi patients as depicted in tab 5.

The findings indicated that VEGF rs699947 variant increased the risk of Breast cancer in codominant (CA vs CC) OR=2.91, 95% CI = 1.18–7.20, P = 0.021) but non-significant for AA vs CC (OR =1.77, 95% CI = 0.97–3.24, P = 0.06) and dominant (CA+AA vs CC) OR = 1.99, 95% CI = 1.13–3.51, P = 0.016) inheritance models tested. During the allelic comparison, the A allele increased the risk of Breast cancer with odd ratio (OR = 1.66, 95% CI = 1.10–2.51, P = 0.020) and risk ratio RR= 1.31(1.04-1.65) P= 0.020) as depicted in Table 5.

Discussion

The study was conducted in a prospective manner, based on a single-institution cohort of Saudi Arabia (Tabuk) women diagnosed with breast cancer. The VEGF rs699947 C/A gene polymorphism was revealed to be associated with an overall increased risk of Breast cancer in different studies. The prevalence of rs699947C/A gene polymorphism was analyzed in the different healthy populations from different and countries of the world as depicted in Table 6. Similarly The prevalence of rs699947 C/A gene polymorphism was analyzed in the Breast cancer women of different populations of the world as depicted in Table 7. The prevalence of rs699947 CA genotype was 45% and AA genotype 18% in Breast cancer patients were identified as significantly higher than that in the healthy individuals (37% and 09%, respectively) and the A allele of rs699947 was found to be as more frequent in patients with Breast cancer than that of controls (0.41 vs 0.28 respectively). We observed a statistically significant difference in the frequencies of VEGF rs699947 genotypes among patients and gender matched healthy controls (p=0.030).

The prevalence of VEGF rs699947 AA genotype in our study group was smaller than that reported in USA (28%), Polish (28%), Swedish (26%), England (25%), and German (22%) except in Morocco (4%) and Thailand (6%) as depicted in Figure 3, whereas the frequency of VEGF rs699947 CA genotype reported in our study group was 45% that was similar to that reported in USA (49%),

Polish (47%), Swedish (47%), England (50%),Thailand and German (49%) as depicted in Figure 3.

VEGF is believed to serve as an important factor for angiogenesis through various mechanisms (Yoshiji et al., 1996). Some of studies identified several functional polymorphisms of the VEGF gene that might affect serum VEGF expression level, including −634G>C, −1154G>A, 936C>T, −1498C>T, −2578C>A, and −460C>T (García-Closas et al., 2007; Koukourakis et al., 2004). Several previous studies reported that functional genetic polymorphisms could alter mRNA or protein expression, thus generating significant influence on disease development of various diseases including cancer (Flego et al., 2013) .

Recently, the relationships between VEGF gene variations and the risk of breast cancer have been extensively studied; however, the reported results were inconsistent. The findings of our study proposed that VEGF rs699947 variant (AA) significantly increased the risk of breast cancer. The stratified analysis was performed by clinicopathological characteristics of breast cancer patients, and our findings proposed that cases with rs699947 AA genotype have increased risk of developing breast cancer in young individuals (age <40 years), as well as in patients with advanced stage. Similarly a significant association was observed with distant metastasis cases. However, patients with CA genotype had a lower risk of developing Progesterone receptor (-ve) and her2-neu negative breast cancer. Similar results were reported by Kapahi et al., (2015) who investigated the impact of VEGF −2578C/A, polymorphisms on Breast cancer in North Indian population. Also Zhang et al., (2015) performed an ethnicity-specific subgroup analysis, but did not find any significant associations between the -2578 C/A polymorphisms in the VEGF gene and breast cancer risk in either Caucasians or Asians. Similarly Chen et al., (2014) performed a meta-analysis revealed no significant association between VEGF −2578C/A and the risk of cancer. Subgroup analyses showed that the VEGF −2578C/A polymorphism is not associated with bladder and breast cancers but is associated with colorectal and lung cancers. A Meta-analysis was performed by Wang et al., (2008) between VEGF SNPS like +936C/T, −1154A/G, −2578C/A, −634G/C, and −460T/C and risk of Breast cancer .The overall results of combined analyses revealed that all the five polymorphisms of VEGF were not associated with the risk of Breast cancer. Nasr et al., (2008) has also shown that −2,578 CA variant correlates with disease stages in which angiogenesis plays a critical role. The VEGF −2,578 C/A polymorphism has shown a significant association with BC susceptibility. Similarly our results showed significant associations of VEGF −2,578CA and −2,578AA genotype with stage status (p=0.042). The distribution of CA and homozygous AA genotype increased significantly with increasing the breast stage among the breast cancer cases (53% Vs 29.72%). However, Rahoui et al., (2014) reported that carriers of −2,578 A allele had a reduced risk to develop a Breast cancer therefore their result is contradicted; however, the results obtained by Scheneider et al., (2008) has shown that AA genotype was associated with higher risk of breast cancer in Caucasian and African-American populations. Meanwhile, Jacobs et al., (2006) have reported that the C allele was associated with increased risk of invasive cancer in the American population, but not for in situ or overall breast cancer. Shahbazi et al., (2002) has shown that the −2,578C allele was associated with increased VEGFA production by peripheral blood mononuclear cells (Cuzick et al., 2006). Many well established and widely used genetic variations that predict increased the risk of breast cancer has been reported (Koukourakis et al., 2004). The elucidation of genetic variations has allowed the testing and FDA approval of a drug which actually decreases the future risk of disease in Breast cancer women at high risk. Unfortunately, the reduction in risk to date has been confined to the ER+ subgroup of tumors. In vitro model suggested a haplotypic effect of the polymorphic VEGFA promoter on both basal and stimulated promoter activity however in non-small cell lung cancer; a low VEGFA expression in cancer tissues was significantly associated with the presence of the −2,578 CC, −634 GG and −1,154 AA and GA genotypes (Cui et al., 2013). The nature of breast carcinogenesis pathways is complex; there is no clear reason for the discrepancies in different studies. Ethnic, genetic, and environmental factors may interact in various ways to affect the risk of Breast cancer in different areas .Several studies have been performed to determine the frequency and distribution of VEGF polymorphism −2,578 A/C in different ethnic groups as summarized inTable 7.

This study investigates the association of −2,578 C/A polymorphisms of VEGF-A with Breast cancer susceptibility and aggressiveness among Saudi women. Thus, characterization of VEGF polymorphisms in healthy women gives for the first time the allelic frequencies of VEGF−2578C/A polymorphism which can be used as a reference in others studies. This case–control study demonstrated that VEGF−2,578 C alleles seem to have a protective effect against Breast cancer in Saudi Arabian women. This genetic variant VEGF−2,578 C may act as a low-penetrance Breast cancer risk gene whereas −2,578 A alleles can be used as genetic biomarkers for Breast cancer susceptibility. The overall results of combined analyses revealed that VEGF −2,578 A allele and A carrier genotypes have been shown to be associated with an increased risk of Breast cancer. This study adds to the emerging evidence that VEGF-A polymorphisms play an important role in Breast cancer development. Further large-scale studies in Saudi population are necessary to confirm our results. Moreover, the effect of other functional VEGF polymorphisms on Breast cancer susceptibility and development merits further surveys. The main limitation of the present study is that it is only of moderate size.

In conclusion, our data showed a significant associations of VEGF -2578C>A polymorphism with BC susceptibility in Saudi women. VEGF -2578AA homozygote significantly increases the risk of Breast cancer and can be useful as predisposing genetic marker for BC. Furthers studies with larger sample sizes are necessary to confirm our findings.

Disclosure

Authors have declared that no competing interests exist.

Consent

All authors hereby declare that all experiments have been examined and approved by the Research ethics committee, University of Tabuk, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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