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Genetic Polymorphisms of Vascular Endothelial Growth Factor (VEGF) in Egyptian Women with Breast Cancer

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ABSTRACT

Background:Vascular endothelial growth factor (VEGF) was considered to have an association with breast cancer because it regulates endothelial cell proliferation, migration and differentiation.

Subjects and methods: One hundred and fifty two women with breast cancer were compared to 100 healthy control Egyptian women recruited from the same locality. VEGF gene polymorphisms were assessed using the PCR-RFLP analysis of DNA samples obtained from peripheral blood.SNP scanning was performed using MnII , BsmfI , CviAII , BsmfI , MnII restriction enzymes for VEGF1154 G/A, 634 G/C, 405 C/G, 936 C/T, 1612 G/A polymorphisms, respectively.

Results : Breast cancer among Egyptian women was strongly associated with the mutations related to VEGF gene polymorphism as follows: VEGF 1154 G allele frequency was significantly higher than the A allele (P = 0.0007,O.R =2.4) , VEGF 634 C allele frequency was significantly higher than the G allele (P = 0.012, O.R =0.62),VEGF 405 C Allele frequency was significantly higher than G Allele (P = 0.009, O.R =1.67), VEGF 936 C Allele frequency was significantly higher than the T Allele (P = 0.0057, O.R =1.72), VEGF 1612 G Allele frequency was significantly higher than A allele (P = 0.0148, O.R =1.62). For VEGF 1154 GA: AA vs. GA+GG (Recessive) P = 0.10, O.R = 6.23, C.I (1.0-38.9), GA vs. AA+GG (over dominant) P= 0.01*, O.R = 2.13, C.I (1.2-3.8),AA+GA vs. GG (dominant) P= 0.0015*, O.R = 2.57, C.I (1.5-4.5). For VEGF 634 GC : CC vs. GC+GG (Recessive) P= 0.1852, O.R = 0.64, C.I (0.4-1.2), GC vs. CC+GG (over dominant) P= 0.2669 , O.R = 0.71, C.I (0.4-1.2), CC+GC vs. GG (dominant) P = 0.0002**, O.R=0.05, C.I (0.0-0.2).For VEGF 405 CG : GG vs. CG+CC (Recessive) P= 0.0013*, O.R = NA,C.I =NA, CG vs. GG+CC (over dominant) P= 0.877, O.R = 1.08, (0.6-1.9), GG+CG vs. CC (dominant) P = 0.0323*, O.R=1.93,C.I (1.1-3.4).For VEGF 936 CT : TT vs. CT+CC (Recessive) P = 0.1833, O.R = 1.63, C.I (0.9-3.1), CT vs. TT+CC (over dominant) P = 0.1379, O.R = 1.55, C.I (0.9-2.6), TT+CT vs. CC(dominant) P = 0.0075**, O.R=2.08, C.I (1.2-3.5). For VEGF 1612 GA: AA vs. GA+GG (Recessive) P = 0.0000**, O.R = NA, C.I = NA, GA vs. AA+GG (over dominant) P= 0.0002**, O.R = 0.36, C.I (0.6-0.2), AA+GA vs. GG (dominant) P = 0.9541, O.R = 0.95, C.I (1.6-0.6).

Key words: Polymorphisms, Breast cancer, VEGF

INTRODUCTION

The breast tissue from the inner lining of milk ducts or the lobules that supply ducts with milk are the main organs that develop the Breast Cancer (malignant breast neoplasm).[1]Lobular carcinomas are cancers of lobules. In the West, the Breast Cancer represents 23% of the total cancer cases within women. It also represents 14% of the total cancer cases in the whole world in 2008 within women. Therefore, the Breast Cancer is the major cancer type among females.[2]In Egypt breast cancer is the most common breast cancer among women representing 18.9% of total cancer cases (35.1% in women) among the Egypt National Cancer Institute (NCI).[3]The mechanism and etiology of breast carcinogenesis remain a mystery. The reasons of having the Breast Cancer are not completely known but they may includehereditary, environmental, dietary, racial and socioeconomic risk factors. They also may includeage at menarche, menopause,genetic reproductive history and estrogen administration factors.[4, 5, 6, 7, 8, 9, 10]The

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inherited mutations explain the minority of the feminine breast cancer cases. However, a combination of common low-penetrance gene polymorphisms exposes the majority of these cases. Tumor growth depends on the essential process of angiogenesis. Angiogenesis supplies routes for tumor dissemination and metastasis.[11, 12]Angiogenesis influences the growth invasion and the formation of metastases. Thus, angiogenesis has a pivotal role in terms of carcinogenesis. In addition, a balance of pro and antiangiogenic regulates the formation of metastases. Vascular endothelial growth factor (VEGF) represents a crucial factor in terms of angiogenesis during the formation of placenta. (VEGF) is over expressed breast cancer.[13]The lymph node and visceral metastasis in different cancers are linked to VEGF.[14, 15, 16] VEGF is found in even the early stages of the breast cancer.[17]There are seven factors, which represent (VEGF). These factors are VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placenta growth factor, and snake venom VEGF (VEGF-F).The surface of endothelial cells contains the receptors of VEGF. The location of the VEGF-A gene is chromosome 6p21.3.[18, 19]It comprises a 14 - kb coding region with eight exons and seven introns[20].

Polymorphisms in the VEGF gene have been shown to be correlated with variations in the production of the VEGF protein.[21, 22, 23, 24, 25]The 1154 G and 634 C alleles have all been associated with increased VEGF expression.[26,27]In this study we investigate the association between the single nucleotide polymorphism (SNPs) in vascular endothelial growth factor (VEGF) gene and risk of breast cancer as up to now a number of studies have reported the association between(VEGF)polymorphism and breast cancer susceptibility but the results remain inconsistent. This study aims at recognizing the genetic variation in vascular endothelial growth factor (VEGF) gene. This helps discovering the early stage of pathogenesis of the breast cancer. The early discovery leads to important advances in prevention of the breast cancer. It also helps knowing the prognostic characteristics of the tumors in the Egyptian cases.

SUBJESTS, MATERIALS AND METHODS:

Studied cases were represented by a cohort sample randomly selected152 Egyptian cases. These cases were taken from the cases presenting with Breast Cancer admitted in the Oncology Center Mansoura University (OCMU) Dakahlia Governorate, Egypt. For analysis of association and risk studies of vascular endothelial growth factor (VEGF), cases were compared to healthy unrelated control subjects of matched age and sex.These cases were questioned about their age, socioeconomic , Education , work, consanguinity, history of diabetes and hypertension,family history of breast cancer, smoking habits, stage, parity, abortion, breast feeding, oral contraceptive usage, nutrition, grade, no of lymph nodes, estrogen, progesterone, site of metastasis.

Statistical Analysis

The data were statistically analyzed by using SPSS program. The Fisher's exact test was performed with Graph pad Instate using the raw data entered into a 2×2 contingency table. Power calculations were performed to give the probability of finding the differences between the gene frequencies as statistically significant, $p<0.05$ was considered as significant, $p<0.01$ was highly significant and $p<0.0001$ was extremely significant. The odds ratio and 95% C.I was also calculated for our patients.

RESULTS

The VEGF genotype and allele frequencies of the control and breast cancer cases are shown for the five VEGF polymorphisms. The genotype distribution of each polymorphism in the patients and controls was in Hardy-Weinberg equilibrium.

DISCUSSION

Previous studies suggested that gene polymorphisms encoding for different inflammatory mediators may represent a susceptibility factor for breast cancer[28,29, 30]Several studies have evaluated the association of VEGF polymorphisms with breast cancer risk.[29, 31]The results however have been inconsistent. Despite the large number of studies with different designs and populations, the role of VEGF gene polymorphisms on breast cancer is still controversial. So, this study aims to determine the relationship between VEGF gene polymorphism and the presence of cancer disease in a sample of Egyptian breast cancer disease patients. In this study we examined VEGF 1154 G/A , 634 G/C , 936 C/T , 405 C/G , 1612 G/A relation with therisk of the breast cancer .Our results were consistent with some previous study.

Regarding the VEGF polymorphism, cases showed significant higher frequency of 1154 A allele, 634 C allele, 405 G allele 936 T allele and 1612 A allele carriage rate that might signify a higher production to the VEGF protein.[28, 32]

In contrast with our results Rani James*et al.*, 2014inIndia showed that for 1154G/A polymorphism, the highest production was observed for the GG genotype, and patients with 1154A allele were showing lowest VEGF production 1154 GA, AA genotypes were significantly less in node positive patients showing an O.R 0.14 & 0.014 respectively .

Furthermorepatient's with1154A allele was showing lowest VEGF production suggesting a protective role of this allele but this was not statistically significant.

However, further studies on larger populations may be necessary to confirm these observations.Schneider*et al.,*2008 in a case control study evaluated the effect 1154GA polymorphism in cancer patients reported an improved median overall survival in patients with VEGF 1154AA genotypes**.**

In conclusion VEGF 1154GA was not related with breast cancer risk.[29, 31, 33, 34]but G allele was related with invasive disease risk when it was high[31, 33, 34]A case–control study, with 571 breast cancer patients, evaluated responsibility of VEGF 1154GA in German individuals, showed that 1154GG has been associated with a higher VEGF production.[29]

In China, Ting luo*et al.,*2013shows that there is an important link between 634 CC genotype and high tumor aggressiveness (large tumor size) (O.R = 2.63, 95% C.I = 1.15-6.02, P = 0.02). The genotypes are not linked with other tumor characteristics, including stage at diagnosis or estrogen or progesterone receptor status and regional or distant metastasis. QianrenJin*et al.*,2005observed an important association among a higher histological grade of tumors, a large tumor size and 634 CC genotype. However these findings agree with many reports examines the effect of the polymorphisms on production of VEGF.The 634CCgenotype has related with higher serum levels of VEGF than GG genotype.Similarly, in agreement with our results, RuhiKapahi*et al.,* 2014in Indiaobserved significantly increased frequency of GG genotype in Cases as Compared to Controls (54.17% vs 38.54% , P = 0.003). A strong association of 405 GG genotype was observed with increased risk for breast cancer (OR = 3.07, 95% CI = 1.41-6.65). In addition, Combined CG and GG genotype wasalso associated with higher breast cancer risk in dominant genetic model (OR = 2.35, 95% CI = 1.12-4.95). He also observed significantly increased frequency of G allele in patients which revealed 1.69 fold higher risk to breastcancer (OR = 1.69, 95% CI = 1.24-2.30, P = 0.0008).

In contrast with our results,inIndiaRaniJames*et al.*,2012 showed that the 405C haplotype that related with promoter activity when it was low. This was more common in healthy women than in patients.He also found that the levels of VEGF were increased significantly (P <0.001) in breast cancer cases compared to healthy women. The highest production was observed for the 405GG genotype, and the lowest for 405CC genotype.

Awata*et al.,*2002reported that individuals with the 405CC genotype had a higher fasting serum VEGF level than those with other genotypes, and that they carried an increased risk of diabetic retinopathy.

There is an important correlation between VEGF protein production and polymorphism 405 C/G or 634 G/C located in potential binding site for MZF1 transcription factor in the 5́ UTR of VEGF.[35]Stevens *et al.,* 2003 also reported that haplotype 405G has a higher promoter activity than haplotype 405C.

In India RuhiKapahi*et al.,* 2014showed that the frequencies CC , CT and TT genotypes of 936 C/T polymorphism were 80.73% vs 89.06% , 18.75% vs 10.42% , 0.52% vs 0.52% in Cases and Controls respectively. There was significantly increased frequency of CT genotype in breast cancer Cases as Compared to Controls (18.75% vs 10.42%, P = 0.021). Individuals carrying CT genotype were associated with two fold risk to breast cancer (OR = 1.99, 95% CI = 1.10-3.58). similarly in agreement with our results he observed that in the dominant model , Individuals carrying the combined CT+TT genotype were significantly associated with 1.94 fold risk for breast cancer compared to CC genotype (OR = 1.94 , 95% CI = 1.09-3.46 , P = 0.023).

Also There was a great difference in the C and T allele frequencies between breast cancer cases and control individuals (P = 0.031). Significantly more frequency of T allele was shown in cases (9.90%) as compared to Controls (5.73%) and individuals

carrying T allele were related with increased risk of developing cancer of breast (O.R = 1.81 , 95% C.I = 1.05-3.12).In Polish women the results found that the 936CT+TT genotypes of VEGF reduced BRCA1-associated breast cancer risk.[36]In (China) A population based case-control study suggested that the polymorphism of 936 CT VEGF may be an available factor for patients with breast cancer among Chinese women.[36]

In Austria a large case-control study in 500 patients with breast cancer and 500 matched healthy control subjects found that the carriers of VEGF 936T allele are at decreased risk for breast cancer.[28]

But some meta-analyses found that the VEGF gene 936 C/T polymorphism may not contribute to breast cancer susceptibility.[37, 38, 39]In China Ting luo*et al.*,2013 showed that the Carriers of 936 T allele (OR = 0.81 , 95% CI = 0.68-0.98, P = 0.03) and 936 TT genotypes (O.R = 0.46 , 95% C.I = 0.28-0.76 , P = 0.002) had a protective effect concerning this disease when stratified by the tumor size, histological grade, stage, regional lymph node, metastasis, distant metastasis, estrogen receptors and progesterone receptor of breast cancer, no statistically significant result was observed .

In china Nobuhiko Kataoka*et al.,* 2006found that women who carry the TT genotype in the C936T polymorphism had a decreased risk of breast cancer among premenopausal women. The C936T polymorphism has been reported to be associated with lower VEGF plasma levels.[22, 40]

Those who are homozygous for TT have lower VEGF production compared with the CC genotype which in turn, may decrease the risk of tumor development.[22]In a previous study among 500 Caucasian breast cancer Cases and 500 Controls, Kripp*et al.,* 2013 have shown a decreased risk of breast cancer in individuals who were 936 T allele carriers.[28]

However, the genotypes in patients did not follow the Hardy-Weinbergequilibrium. In another study no association between the 936 polymorphism and risk to breast cancer among 862 Cases and 713 Controls could be observed.[41]

QianrenJin*et al.,* 2005 observed no differences in the allele or genotype frequencies between either the familial or unselected breast cancer case and respective control group nor did the joint analyses show any differences between the cases and controls (odds ratio = 0.99 , 95% CI = 0.85 – 1.15 , P = 0.93).

He provided strong evidence that the 936 T allele or the other studied polymorphisms do not modify the risk of breast cancer and this result was not surprising because VEGF as a key mediator of angiogenesis is more likely to alter the aggressiveness of the tumor than susceptibility to cancer. In agreement with our results in China, Ting luo*et al.,*2013shows that there is no possible correlation between 1612 G/A polymorphism and breast cancer risk. If the classification depends on the progesterone receptor of breast cancer, estrogen receptors, distant metastasis, metastasis, regional lymph node, stage,histologicalgrade and tumor size, there is no important result.

Conclusion: There is a great probability association of VEGF polymorphism with the occurrence of breast cancer among Egyptian cases, Regarding the VEGF polymorphism, The cases in this study showed that there were a significant higher frequency of 1154 A allele, 634 C allele, 405 G allele 936 T allele and 1612 A allele carriage rate that might signify a higher production to the VEGF protein In Egyptian population.

PCR is a relatively simple and accurate method for detection of VEGF polymorphism in breast cancer cases.

Therefore we recommend: Recognition and characterization of VEGF polymorphism by polymerase chain reaction (PCR) can help in diagnosis of susceptible cases for early discovery and prevention of breast cancer diseases among affected families, Routine screening for breast cancer mutations for all Egyptian women in order to setup an appropriate method of prophylaxis against breast cancer disorder , We also recommended for proper environmental behavior, combating pollution and stopping the bad health habits like smoking to protect individuals carrying the unfavorable genes making them susceptible to breast cancer.

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PCR amplification

polymorphism	*Primers*	*Conditions of PCR*	*PCR Product*	*Restriction enzymes*	*Fragments after digestion*
1154 G/A	(F5'-TCCTGCTCCCTCCTCGCCAATG-3') (R5'-GGCGGGGACAGGCGAGCATC-3')	5 minutes at 95°c, followed by 35 cycles of denaturation at 94° c for 45 seconds, annealing at 60°c for 45 seconds , and extension at 72° c for 45 seconds, followed by a final extension at 72° c for 7 minutes	206 bp	MnlI	(G) 150 bp , 34 bp , 22 bp&(A) 184 bp , 22 bp
634 G/C	(F5'-ATTTATTTTTGTCTGTCTGTCTGTCCGTCA-3') (R5'-TAGGCCAGACCCTGGCAC-3')	94°c for 5 minutes (35 cycles) then denatured for 40 seconds at 94° c, annealed for 60 seconds at 58°c, and extension for 40 seconds at 72° c , finally extension for 7 minutes at 72° c.	304 bp	BsmfI	(G) 193 bp , 111 bp &(C) 304 bp
936 C/T	(F5'-CTCGGTGATTTAGCAGCAAG-3') (R5'-CTCGGTGATTTAGCAGCAAG-3')	94°c for 5 minutes (35 cycles) then denatured for 30 seconds at 94° c, annealed for 60 seconds at 62°c, and extension for 30 seconds at 72° c, finally extension for 10 minutes at 72° c.	198 bp	CviAII	(C) 198 bp&(T) 112 bp , 86 bp
405 C/G	(F5'-ATTTATTTTTGCTTGCCATT-3') (R5'-GTCTGTCTGTCTGTCCGTCA-3')	10 minutes at 95°c, followed by 35 cycles of denaturation at 95° c for 45 seconds, annealing at 62°c for 45 seconds , and extension at 72° c for 40 seconds, followed by a final extension at 72° c for 7 minutes.	197 bp	BsmfI	(G) 167 bp , 30 bp&(C) 197 bp
1612 G/A	(F5'-CACATGCTGCACGCGCATCTCA-3') (R5'-ACCCCAGGAAGGGGAGCAGGA-3')	94°c for 5 minutes (35 cycles) then denatured for 30 seconds at 94° c, annealed for 60 seconds at 62°c, and extension for 30 seconds at 72° c , finally extension for 10 minutes at 72° c.	206 bp	MnlI	(G) 150 bp , 34 bp , 22 bp (A) 184 bp , 22 bp

Then 17 µldistwater, 3 µlbuffersfor each restriction enzyme then PCR products were incubated over night at 37°c for (16 – 24 hours) and the digested products were detected on a 3% ethidium bromide agarose gel and visualized under UV light.

Table1. Genotype frequencies of VEGF polymorphisms in a sample of Egyptian women with breast cancer compared to controls.

VEGF 1154 GA	Cases n = 152 (%)	Controls n = 100 (%)
GG	86 (56.6%)	77 (77.0%)
GA	57 (37.5%)	22 (22.0%)
AA	9 (5.9%)	1 (1.0%)
HWE	$\chi2$= 0.012, p>0.05	$\chi2$=0.174, p>0.05
Allele G	229	176
Allele A	75	24
VEGF 634 GC		
GG	25 (16.4%)	1 (1.0%)
GC	98 (64.5%)	72 (72.0%)
CC	29 (19.1%)	27 (27.0%)
HWE	$\chi2$=12.82, p<0.01	$\chi2$=29.64, p<0.01
Allele G	148	74
Allele C	156	126
VEGF 405 CG		
CC	32 (21.1%)	34 (34.0%)
CG	103 (67.8%)	66 (66.0%)
GG	17 (11.2%)	0 (.0%)
HWE	$\chi2$=20.65, p<0.01	$\chi2$=24.26, p<0.01
Allele C	167	134
Allele G	137	66
VEGF 936 CT		
CC	52 (34.2%)	52 (52.0%)
CT	64 (42.1%)	32 (32.0%)
TT	36 (23.7%)	16 (16.0%)
HWE	$\chi2$=3.35, p<0.01	$\chi2$=7.01, p<0.01
Allele C	168	136
Allele T	136	64
VEGF 1612 GA		
GG	55 (36.2%)	35 (35.0%)
GA	61 (40.1%)	65 (65.0%)
AA	36 (23.7%)	0 (.0%)
HWE	$\chi2$=5.18, p<0.01	$\chi2$=23.18, p<0.01
Allele G	171	135
Allele A	133	65

Table2. Genotype frequencies of VEGF polymorphisms in a sample of Egyptian women with breast cancer compared to controls.

Inheritance model	Statistics	p	OR, 95% C.I
	VEGF 1154 GA		
Genotypic	**GG vs. GA vs. AA**	0.002*	
Recessive	**AA vs. GA+GG**	0.10	6.23, (1.0-38.9)
over dominant	**GA vs. AA+GG**	0.01*	2.13, (1.2-3.8)
dominant	**AA+GA vs. GG**	0.0015*	2.57, (1.5-4.5)
Allelic	**A vs. G**	0.0007**	2.4, (1.5-3.9)
	VEGF 634 GC		
Genotypic	**GG vs. GC vs. CC**	.000**	
Recessive	**CC vs. GC+GG**	0.1852	0.64, (0.4-1.2)
over dominant	**GC vs. CC+GG**	0.2669	0.71, (0.4-1.2)
dominant	**CC+GC vs. GG**	0.0002**	0.05, (0.0-0.2)
Allelic	**C vs. G**	0.0127*	0.62, (0.4-0.9)
	VEGF 405 CG		
Genotypic	**CC vs. CG vs. GG**	.001*	
Recessive	**GG vs. CG+CC**	0.0013*	NA
over dominant	**CG vs. GG+CC**	0.877	1.08, (0.6-1.9)
dominant	**GG+CG vs. CC**	0.0323*	1.93, (1.1-3.4)
Allelic	**G vs. C**	0.0091*	1.67, (1.2-2.4)
	VEGF 936 CT		
Genotypic	**CC vs. CT vs. TT**	0.019*	
Recessive	**TT vs. CT+CC**	0.1833	1.63, (0.9-3.1)
over dominant	**CT vs. TT+CC**	0.1379	1.55, (0.9-2.6)
dominant	**TT+CT vs. CC**	0.0075**	2.08, (1.2-3.5)
Allelic	**T vs. C**	0.0057**	1.72, (1.2-2.5)
	VEGF 1612 GA		
Genotypic	**GG vs. GA vs. AA**	.000**	
Recessive	**AA vs. GA+GG**	0.0000**	NA
over dominant	**GA vs. AA+GG**	0.0002**	0.36, (0.6-0.2)
dominant	**AA+GA vs. GG**	0.9541	0.95, (1.6-0.6)
Allelic	**A vs. G**	0.0148*	1.62, (1.1-2.3)

Table3.Logistic regression of demographic and clinical variables in relation to genotypes.

Demographic and clinical variables	**VEGF1154 GG vs. GA+AA**
	P, O.R (95% C.I.)
age	0.486, 1.012 (0.979-1.046)
Family history	0.479, 0.698 (0.257-1.891)
consanguinity	0.300, 0.662 (0.253-1.527)
smoking	0.648, 0.848 (0.418-1.719)
BMI	0.849, 0.993 (0.922-1.069)
Age at menstruation	0.977, 1.005 (0.732-1.378)
parity	0.345, 0.899 (0.721-1.121)
First pregnancy	0.560, 1.019 (0.955-1.088)
Breast feeding (1)	0. 337, 1.794 (0.544-5.911)
Breast feeding (2)	0. 699, 1.303 (0.341-4.973)
Menopause (1)	0.848, 0.930 (0.444-1.949)
Oral contraceptive	0.098, 1.912 (0.887-4.121)
estrogen	0.277, 4.346 (0.307-1.442)
progesterone	0.466, 0.375 (0.027-5.228)
Demographic and clinical variables	**VEGF 634 GG vs. GC+CC**
	P, O.R (95% C.I.)
age	0.205, 0.971 (0.928-1.016)
Family history	0.757,0.818 (0.229-2.924)
consanguinity	0.782,0.847 (0.260-2.758)
smoking	0.157,0.478 (0.172-1.327)
BMI	0.678,1.022 (0.923-1.131)
Age at menstruation	0.582,1.129 (0.733-1.739)
parity	0.160,1.250 (0.916-1.705)
First pregnancy	0.425,1.036 (0.950-1.129)
Breast feeding (1)	0.168,0.384 (0.098-1.500)
Breast feeding (2)	0.960,1.049 (0.165-6.684)
Menopause (1)	0.379,1.585 (0.568-4.420)
Oral contraceptive	0.827,0.890 (0.312-2.539)
estrogen	0.687,0.464 (0.011-19.321)
progesterone	0.556,3.069 (0.073-128.574)
Demographic and clinical variables	**VEGF 405 CC vs. CG+GG**
	P, O.R (95% C.I.)
age	0.090,1.037 (0.994-1.081)
Family history	0.500,1.591 (0.413-6.130)
consanguinity	0.970,1.021 (0.351-2.968)
smoking	0.804,0.898 (0.383-2.104)
BMI	0.773,0.987 (0.905-1.077)
Age at menstruation	0.825,1.046 (0.702-1.558)
parity	0.391,0.891 (0.685-1.159)
First pregnancy	0.918,1.005 (0.922-1.094)
Breast feeding (1)	0.853,1.143 (0.277-4.711)
Breast feeding (2)	0.487,1.915 (0.306-11.991)
Menopause (1)	0.961,1.023 (0.414-2.531)
Oral contraceptive	0.232,1.729 (0.705-4.238)
estrogen	0.852,0.778 (0.056-10.775)
progesterone	0.678,1.751 (0.125-24.614)
Demographic and clinical variables	**VEGF 936 CC vs. CT+TT**
	P, O.R (95% C.I.)
age	0.368 , 1.017 (0.981-1.054)
Family history	0.679, 0.801, (0.280-2.288)
consanguinity	0.154, 2.066, (0.762-5.599)
smoking	0.271, 0.655, (0.308-1.392)
BMI	0.958, 0.998, (0.923-1.079)
Age at menstruation	0.754, 1.056, (0.751-1.484)
parity	0.205, 1.175, (0.916-1.508)
First pregnancy	0.186, 0.955, (0.892-1.022)

Breast feeding (1)	0.534, 1.510 , (0.412-5.533)
Breast feeding (2)	0.203, 0.404, (0.100-1.628)
Menopause (1)	0.413, 0.719, (0.327-1.583)
Oral contraceptive	0.324, 0.664, (0.294-1.498)
estrogen	0.178, 5.899, (0.445-78.148)
progesterone	0.237, 0.206 , (0.015-2.833)
Demographic and clinical variables	**VEGF 1612 GG vs. GA+AA P, O.R (95% C.I.)**
age	0.344, 1.018, (0.981-1.057)
Family history	0.561, 1.405, (0.447-4.417)
consanguinity	0.023, 3.281, (1.174-9.171)
smoking	0.163, 0.573, (0.262-1.254)
BMI	0.188, 1.056, (0.974-1.146)
Age at menstruation	0.026, 0.657, (0.455-0.950)
parity	0.768, 1.038, (0.810-1.330)
First pregnancy	0.016, 0.910, (0.842-0.982)
Breast feeding (1)	0.272, 0.494 , (0.141-1.738)
Breast feeding (2)	0.853, 0.870, (0.199-3.805)
Menopause (1)	0.554 , 0.779, (0.340-1.783)
Oral contraceptive	0.902, 0.950, (0.421-2.145)
estrogen	0.767, 1.486,(0.109-20.321)
progesterone	0.747, 0.649, (0.047-8.992)

Table4. Summary of reported studies showing the polymorphism of VEGF 936 C/T in different Ethnic Groups.

PCR-RFLP: Polymerase chain reaction – restriction fragment length polymorphism, SSCP : Single strand conformation polymorphism

variant	Cases /Controls	Method	Ethnic population	Inference	Reference
936 C/T					
	152/100	PCR-RFLP	Egyptian	there were a significant higher frequency of 936 T allele	**Present study**
	192/192	PCR-RFLP	North Indian	Association of CT and CT+TT genotype with increased breast cancer risk	**Ruhi*et al.*, 2014**[32]
	500/500	PCR-RFLP	Austrian	Carriers of T allele associated with the decreased breast cancer risk	**Kripp*et al.*, 2003**[28]
	412/422	PCR-RFLP	Polish	No significant association	**Jin*et al.*, 2005**[42]
	153/163	PCR-RFLP	German	No significant association	
	924/934	TaqMan	Swedish	No significant association	
	501/504	TaqMan	-	Association of CC genotype with decreased risk for in situ breast cancer	**Jacobs *et al.*, 2006**[31]
	1109/1195	TaqMan	Chinese	Association of TT genotype with the decreased breast cancer risk	**Kataoka*et al.*, 2006**[36]
	848/702	TaqMan	Caucasians	No significant association	**Balasubramanian*et al.*, 2007**[43]
	500/500	PCR-RFLP	Austrian	Association of T allele with decreased breast cancer risk	**Gerger*et al.*, 2007**[44]
	60/60	SSCP	Turkish	Significantly increased frequency of CT genotype among patients	**Erogl*et al.*, 2008**[45]
	319/290	PCR-RFLP	Polish	Association of CT+TT genotype with decreased breast cancer risk	**Jakubowska*et al.*, 2008**[35]
	804/804	TaqMan	Austrian	No significant association	**Langsenlehner*et al.*, 2008**[46]
	520/715	TaqMan	Caucasians	No significant association	**Schneider *et al.*, 2008**[33]
	235/235	PCR-RFLP	Brazilian	No significant association	**Oliveira *et al.*, 2011**[47]
	1918/1819	TaqMan	Chinese	No significant association	**Zhang *et al.*, 2011**[48]
	453/461	TaqMan& PCR-RFLP	Spanish	Association of CT+TT genotype with decreased breast cancer risk	**Rodrigues *et al.*, 2012**[49]
	680/680	PCR-RFLP	Chinese	Association of T allele with decreased breast cancer risk	**Luo*et al.*, 2013**[50]

Table5. Summary of reported studies showing relationship of VEGF 405 C/G Polymorphism and breast cancer risk in different Ethnic Groups.

variant	Cases /Controls	Method	Ethnic population	Inference	Reference
405 C/G					
	152/100	PCR-RFLP	Egyptian	there were a significant higher frequency of 405 G allele	Present study
	192/192	PCR-RFLP	North Indian	Association of CG and CG+GG genotype with increased breast cancer risk	Ruhi*et al.*, 2014[32]
	501/504	TaqMan	-	No significant association	Jacobs *et al.*, 2006[31]
	936/941	TaqMan	Swedish	No significant association	Jin*et al.*, 2005[42]
	1095/1198	TaqMan	Chinese	No significant association	Kataoka*et al.*, 2006[36]
	490/498	TaqMan	Caucasians	No significant association	Balasubramanian*et al.*, 2007[43]
	804/804	TaqMan	Austrian	No significant association	Langsenlehner*et al.*, 2008[46]
	520/715	TaqMan	Caucasians	No significant association	Schneider *et al.*, 2008[33]
	235/235	PCR-RFLP	Brazilian	Association of CC genotype with the increased breast cancer risk	Oliveira *et al.*, 2011[47]
	680/680	PCR-RFLP	Chinese	No significant association	Luo*et al.*, 2013[50]

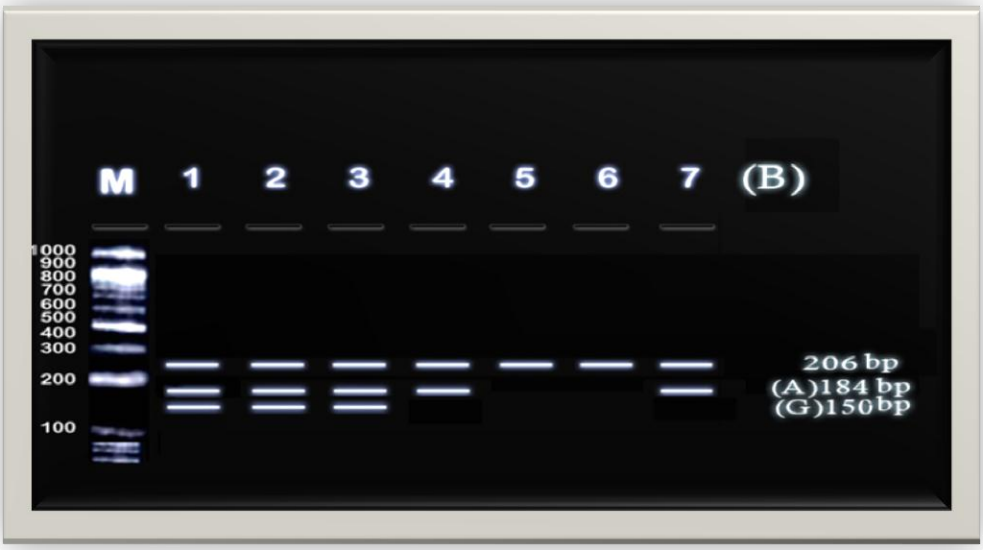

Fig. (1): Amplification of VEGF in 1154 G/A polymorphism in a sample of breast cancer cases , PCR amplicons of 1154 G/A polymorphism amplification product (206 bp) , M : Molecular marker , bp : base pair

(b) Restriction digested products of 1154 G/A polymorphism (Lanes 1, 2, 3 are heterozygous (GA), Lanes 4, 7 are homozygous Mutant (AA), and Lanes 5, 6 are homozygous normal (wild type) (GG).)

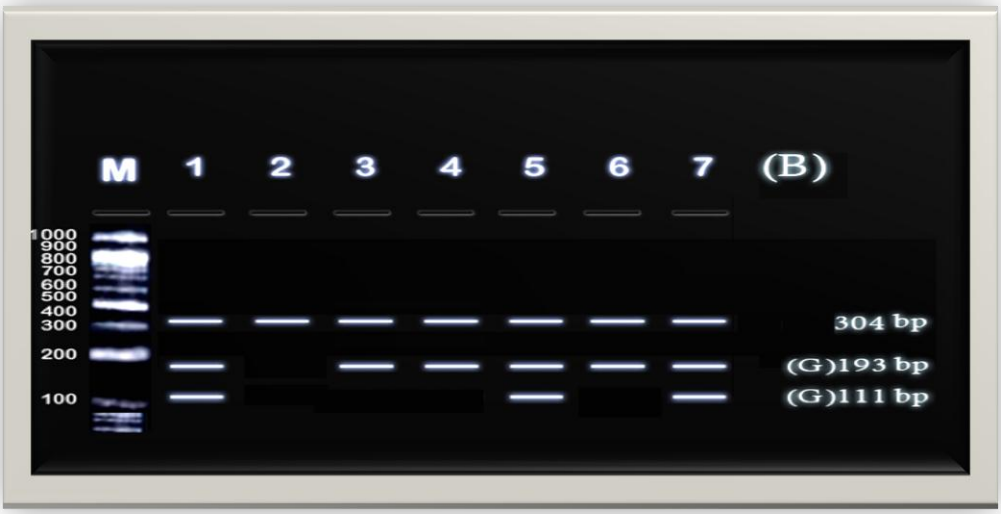

Fig. (2): Amplification of VEGF in 634 G/C polymorphism in a sample of breast cancer cases , PCR amplicons of 634 G/C polymorphism amplification product (304 bp) , M : Molecular marker , bp : base pair

(b) Restriction digested products of 634 G/C polymorphism (Lanes 1, 5, 7 are heterozygous (GC), Lane 2 is homozygous Mutant (CC), and Lanes 3, 4, 6 are homozygous normal (wild type) (GG).)

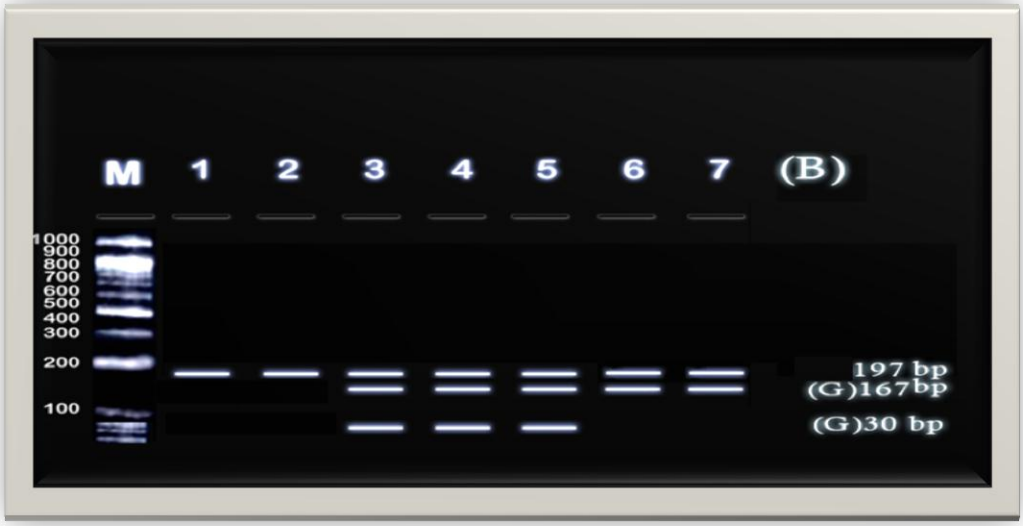

Fig. (3): Amplification of VEGF in 405 C/G polymorphism in a sample of breast cancer cases, PCR amplicons of 405 C/G polymorphism amplification product (197 bp) , M : Molecular marker , bp : base pair

(b) Restriction digested products of 405 C/G polymorphism (Lanes 3, 4, 5 are heterozygous (CG), Lanes 6, 7 are homozygous Mutant (GG), and Lanes 1, 2 are homozygous normal (wild type) (CC).)

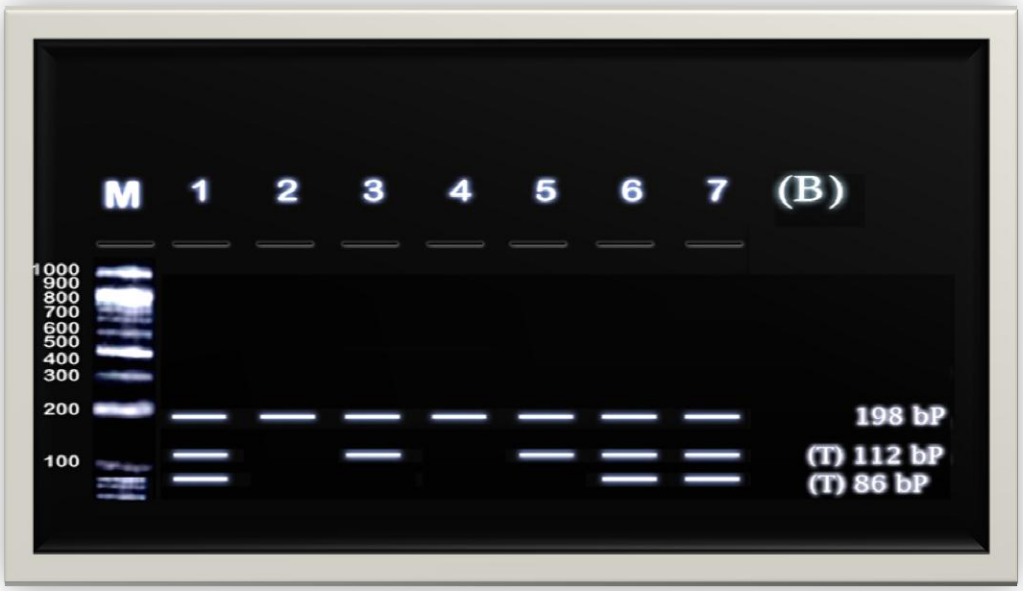

Fig. (4): Amplification of VEGF in 936 C/T polymorphism in a sample of breast cancer cases , PCR amplicons of 936 C/T polymorphism amplification product (198 bp) , M : Molecular marker , bp : base pair

(b) Restriction digested products of 936 C/T polymorphism (Lanes 1, 6, 7 are heterozygous (CT), Lane 3, 5 are homozygous Mutant (TT), and Lanes 2, 4 are homozygous normal (wild type) (CC).)

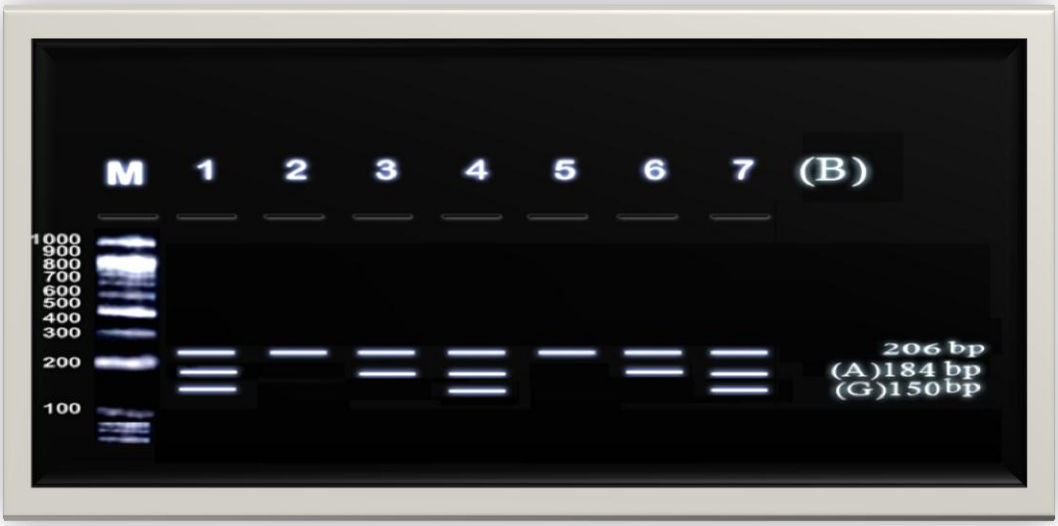

Fig. (5): Amplification of VEGF in 1612 G/A polymorphismin a sample of breast cancer cases, PCR amplicons of 1612 G/A polymorphism amplification product (206 bp), M : Molecular marker , bp : base pair

(b) Restriction digested products of 1612 G/A polymorphism (Lanes 1, 4, 7 are heterozygous (GA), Lanes 3, 6 are homozygous Mutant (AA), and Lanes 2, 5 are homozygous normal (wild type) (GG).)