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RESEARCH

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Association of *VEGFR1*, *VEGFR2* and *VEGFR3* polymorphisms with esophageal cancer risk: a case–control study

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Abstract

Background Esophageal cancer is the eleventh most common cancer and is the seventh leading cause of mortality worldwide. Vascular endothelial growth factor (VEGF) and its receptors pathway are a key regulator of angiogenesis and play an important role in carcinogenesis. The aim of current study was to evaluate the association of five *VEGFR* polymorphisms with esophageal cancer risk in patients from Punjab, North-west India.

Methods This case–control study included 310 esophageal cancer patients and 325 age and gender matched healthy controls. *VEGFR1*-710C/T, *VEGFR2*-604 T/C (rs2071559), *VEGFR2* 1192 G/A (rs2305948), *VEGFR2* 1719A/T (rs1870377) and *VEGFR3* (rs72816988) polymorphisms were genotyped by using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. Restriction digestion products were analyzed on 2.4% agarose gel and genotype was assigned to each sample on the basis of fragments obtained after digestion. Randomly 10% samples were repeated by Sanger sequencing to revalidate the results.

Results There was a significant association of CT genotype (OR = 0.28; 95%CI, 0.10–0.76; $p = 0.01$) and T allele (OR = 0.28; 95%CI, 0.10–0.77; $p = 0.01$) of *VEGFR1*-710C/T polymorphism with decreased risk of esophageal cancer. TC genotype of *VEGFR2*-604 T/C (OR = 0.66; 95%CI, 0.44–0.97; $p = 0.03$) and GA genotype of *VEGFR2* 1192G/A (OR = 0.54; 95%CI, 0.31–0.95; $p = 0.03$) polymorphisms were significantly associated with decreased risk of esophageal cancer. There was no significant difference in allele and genotype frequency of *VEGFR2* 1719A/T and *VEGFR3* (rs72816988) polymorphisms between esophageal cancer patients and controls ($p > 0.05$). Haplotype analysis revealed that haplotype $C\text{-}_{604}A_{1719}A_{1192}$ was significantly associated with the decreased esophageal cancer risk (OR = 0.44; 95%CI, 0.23–0.84; $p = 0.01$).

Conclusion *VEGFR1*-710C/T, *VEGFR2*-604 T/C and *VEGFR2* 1192G/A polymorphisms were associated with the decreased risk of esophageal cancer in patients from Punjab, North-west India.

Keywords Polymorphism, Esophageal cancer, Angiogenesis, *VEGFRs*

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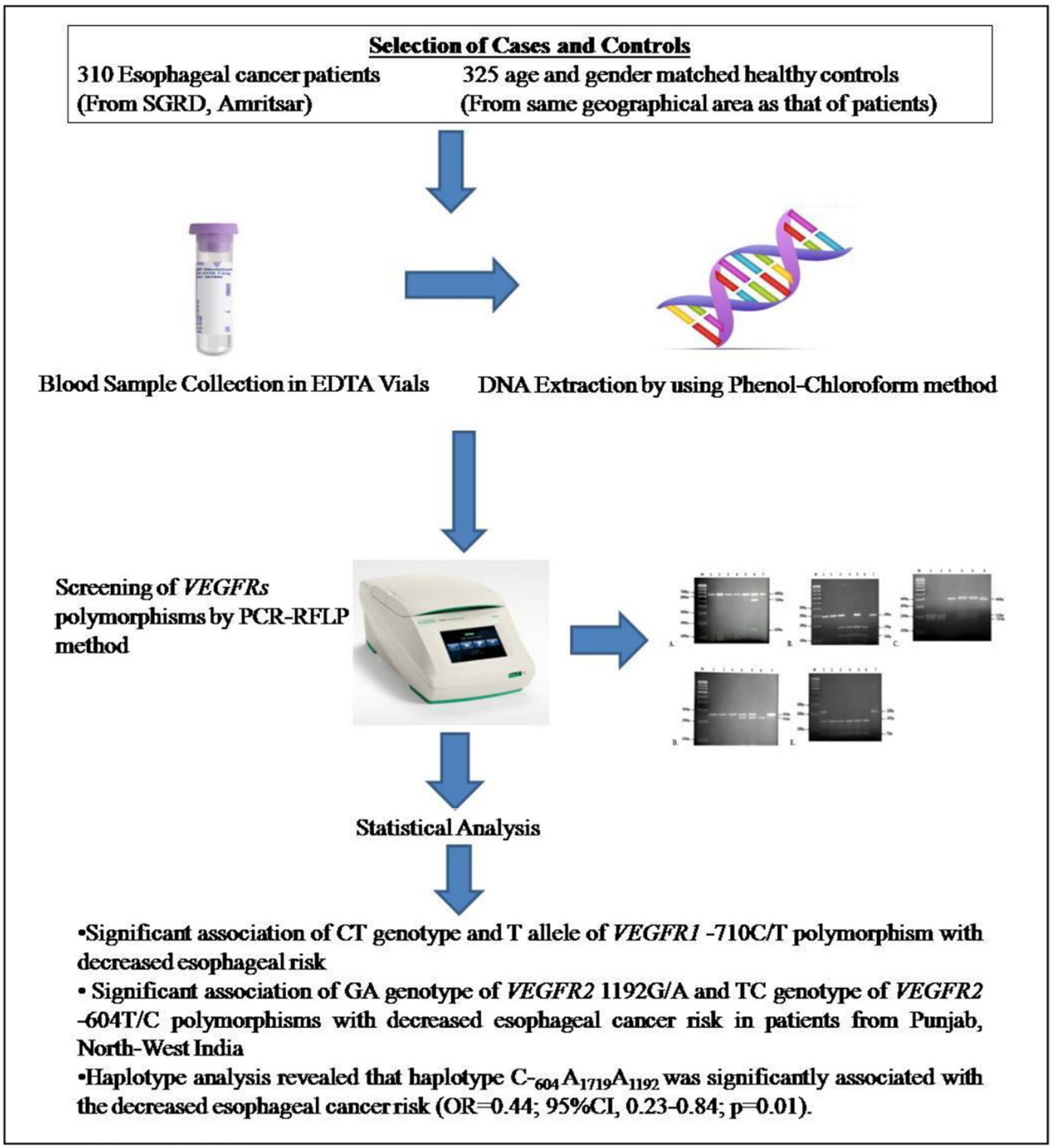
Graphical abstract



Background

The growth of solid tumors including esophageal cancer depends on angiogenesis for the supply of oxygen and nutrients for their continuous growth. Angiogenesis is regulated by cellular signaling mediated by vascular endothelial growth factor (VEGF) and its receptors [1, 2]. VEGF triggers its signaling via VEGFR1, VEGFR2 and VEGFR3 receptors, which are the members of

receptor tyrosine kinase family. It has been reported that VEGFR1 or FLT had greater affinity for VEGF as compared to VEGFR2, but had lower tyrosine kinase activity [3–5]. VEGFR1 is one of the important receptors of VEGF angiogenesis signaling pathway and its expression is upregulated by hypoxia via HIF-1-dependent mechanism [6, 7]. VEGFR2 or KDR had higher affinity for VEGF-A and VEGF-E and lower affinity for VEGF-C and VEGF-D [8, 9]. von Willebrand factor is secreted by endothelial cells when VEGF binds with VEGFR2 and was reported to be one of the negative prognostic factors for many solid tumors [3, 10]. It has been documented that VEGF-VEGFR2 signaling cascade facilitates tumor growth, invasion and therapeutic resistance [11]. VEGFR1 and VEGFR2 have been described as major therapeutic targets for sorafenib [2]. VEGFR3 or FLT-4 has an affinity for VEGF-C and VEGF-D and its expression influenced the differentiation of lymphatic endothelial cells, tubulogenesis, proliferation, migration and survival of lymphatic endothelial cells [3, 8].

Biomarkers like single-nucleotide polymorphisms (SNPs) account for much of the genetic variations including disease susceptibility, prognosis and response to therapy. The angiogenic pathway, and hence the susceptibility and severity of cancer, may be affected by polymorphisms alone or in combination with environmental factors [12]. It has been reported that SNPs in *VEGFRs* may affect the production and functioning of protein, thus resulting in dysregulation of angiogenic pathway [13]. Several SNPs have been identified in the *VEGFR2*, some of which have the ability to alter gene expression, amount of circulating VEGFR2 levels and the efficiency with which VEGF binds to the receptor [14]. Genetic location of the *VEGFR1, VEGFR2 and VEGFR3* polymorphisms is given in Fig. 1.

Association of *VEGFR1, VEGFR2 and VEGFR3* polymorphisms with risk of some of gastrointestinal tract (GIT) cancers has been reported in different populations. The G allele of *VEGFR2*-604A/G polymorphism was associated with increased risk of pancreatic cancer in Romanian population [15]. Combined TT + TC genotype of *VEGFR2*-604 T/C polymorphism was associated with improved overall survival in Danish colorectal cancer patients [16]. The CC genotype of *VEGFR2* 1192 T/C polymorphism was associated with improved survival in Danish colorectal cancer patients [16]. In Han Chinese population, TC genotype of *VEGFR2* 1192 T/C polymorphism was associated with low overall survival in hepatocellular cancer patients [17]. The T allele of *VEGFR2* 1416A/T polymorphism was found to be associated with increased risk of hepatocellular carcinoma in Portuguese population [18]. In Chinese gastric cancer patients, AA genotype of *VEGFR2* 1719 A/T polymorphism was associated with poor prognosis [19]. So far, there is no published study that has investigated the role of *VEGFR1*-710C/T and *VEGFR3* rs72816988 polymorphisms in any of the GIT cancers.

Esophageal cancer is the eleventh most common cancer and is the seventh leading cause of mortality worldwide [20]. The highest regional standardized incidence and mortality of esophageal cancer was found in Eastern Asia, followed by Eastern Africa, Southern Africa and South-Central Asia [21]. According to Globocan 2020, nearly 79.7% of new esophageal cancer cases were found in Asia [22]. China had the largest number of new esophageal cancer cases, accounting for 67.3% of in Asia

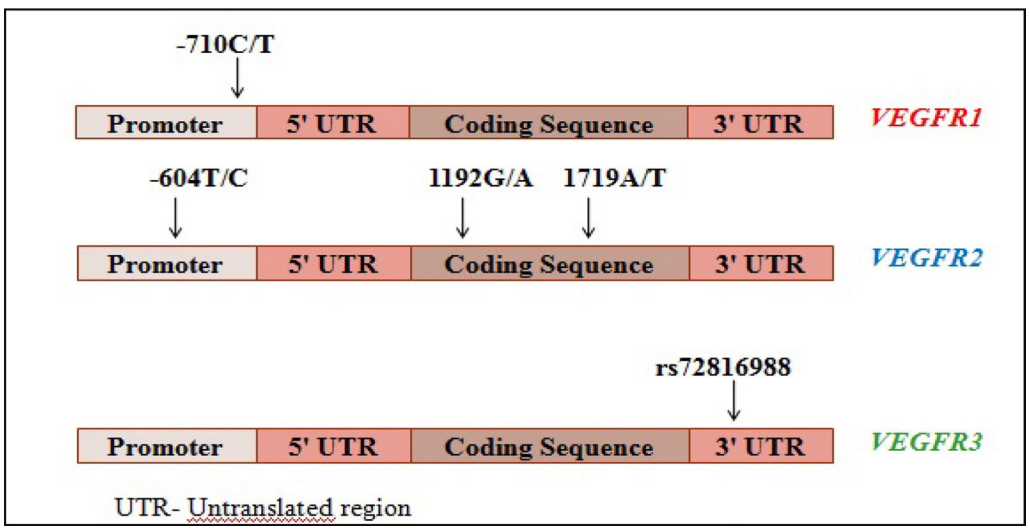


Fig. 1 Genetic location of the screened polymorphisms

and 53.70% of cases worldwide, and India has the second highest number of new esophageal cancer cases in both Asia and the world, with 63,180 new cases [22]. In Punjab, esophageal cancer is the fourth leading cause of death in women and the second leading cause of mortality in men [23]. Histologically, esophageal cancer has two main subtypes, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), and they both differ in their incidence and risk factors profiling. Potential risk factors that one may have for being diagnosed with EAC are obesity, gastroesophageal reflux disease (GERD), male sex, white race and cigarette smoking (or a history of smoking) [24]. For ESCC, the potential risk factors are black race, smoking, alcohol drinking, diet rich in tea, coffee, tobacco chewing, and "chewers of areca nut" which is commonly consumed in regions such as Southeast Asia and India [25].

VEGF/VEGFR pathway is the key regulator of angiogenesis and plays an important role in carcinogenesis [26]. From the early phases of carcinogenesis to the final stage of the disease, angiogenesis plays a significant role in esophageal cancer, and angiogenesis-related agents are being investigated as potential targets for new treatments for esophageal cancer [27]. Therefore, the present study aimed to evaluate the association of *VEGFR1*-710C/T, *VEGFR2*-604 T/C (rs2071559), *VEGFR2* 1192 G/A (rs2305948), *VEGFR2* 1719A/T (rs1870377) and *VEGFR3* (rs72816988) polymorphisms with esophageal cancer risk in patients from Punjab, North-west India. Identification of association of SNPs can aid in predicting the clinical response to the various therapeutic drugs used in the treatment of esophageal cancer. To best of our knowledge, this is the first study evaluating the association of five *VEGFR* polymorphisms with esophageal cancer risk.

Material and methods

Study subjects

The present case–control study was carried out in accordance with the guidelines of the Helsinki Declaration and was approved by the ethics committee of Guru Nanak Dev University, Amritsar, Punjab, India. In this case–control study design, 310 esophageal cancer patients (137 males and 173 females) and 325 (144 males and 181 females) age and gender matched healthy controls from same ethnicity were investigated based on the predefined inclusion and exclusion criteria (Table 1). The sample size was calculated by using online software Cats Power Calculator (https://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html) using data of minor allele frequency of *VEGFR1*, *VEGFR2* and *VEGFR3* polymorphisms from dbSNP (1000 Genome Data). The threshold for significance was set at 0.05, and relative risk was set at 1.5. Five milliliter intravenous blood sample of each subject was collected in EDTA vials after obtaining the written informed consent from all the subjects. The patients were investigated at Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India. The blood samples were transported to the Department of Human Genetics, Guru Nanak Dev University, Amritsar, in an ice box from the site of sample collection. Unique code was given to each sample and was stored at − 20℃ till further processing. Demographic characteristics of study participants are shown in Table 2.

Genomic DNA extraction and genotyping of VEGFR polymorphisms

Genomic DNA was extracted from blood samples using standard phenol chloroform method with few modifications [28]. The procedure of DNA extraction is given in Supplementary file 1. Quantity and quality of DNA samples was analyzed on 1% agarose gel. *VEGFR1*-710C/T, *VEGFR2*-604 T/C (rs2071559), *VEGFR2* 1192 G/A (rs2305948), *VEGFR2* 1719A/T or 1416A/T (rs1870377) and *VEGFR3* (rs72816988) polymorphisms were screened by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method (Figs. 2, 3, 4, 5, 6).

The targeted regions of *VEGFR1, VEGFR2* and *VEGFR3* were amplified using published primer sequence [29–31]. The reaction volume used for amplification was 15 µl,

Table 1 Inclusion and exclusion criteria for case and controls

Inclusion criteria	Exclusion criteria
Patients	
Preoperative clinically confirmed esophageal cancer patients No previous history of any other cancer	Patients who had received chemotherapy, radiotherapy or blood transfusions within three months from the sample's collection data Patients with any infectious disease like HIV, hepatitis
Controls	
Age and gender matched unrelated healthy individual from same geographical are as that of patients No history of any cancer or any chronic disease from last three generations	Individual suffering from any chronic disease or infectious disease like HIV, hepatitis On regular medication

Table 2 Demographic characteristics of the study participants

Variable	Patients n (%)	Controls n (%)
Total	310	325
Male	137 (44.19)	144 (44.31)
Female	173 (55.81)	181 (55.69)
Mean age (Years)		
Total	56.61 ± 13.07	53.13 ± 13.47
Male	58.57 ± 13.38	56.51 ± 13.74
Female	55.06 ± 12.61	53.82 ± 13.25
Diet		
Veg	162 (52.26)	179 (55.08)
Non-veg	148 (47.74)	146 (44.92)
Habitat		
Urban	53 (17.10)	57 (17.54)
Rural	246 (79.35)	255 (78.46)
Suburban	11 (3.55)	13 (4.0)
Smoking status		
Smokers	44 (14.19)	11 (3.38)
Non-smokers	266 (85.81)	314 (96.62)
Alcohol consumption		
Yes	82 (26.45)	72 (22.15)
No	228 (73.55)	253 (77.85)
Type of cancer		
Squamous cell carcinoma (SCC)	273 (88.07)	–
Adenocarcinoma (AC)	29 (9.35)	
Status unknown	8 (2.58)	

and it contained 75 ng of template DNA, 1.5 µl of 10X *Taq* Buffer A with 15 mM $MgCl_2$, 6 pmol of each primer, 0.3 µl of dNTPs mixture and 1 U *Taq* DNA polymerase. Amplified PCR products were analyzed on 2% agarose gel. PCR products were further digested with specific restriction enzymes as per manufacturer's instructions. For *VEGFR1*-710C/T polymorphism, *Nla*III restriction enzyme (New England BioLabs) was used to digest the 665-bp PCR products. For *VEGFR2*-604 T/C polymorphism, *Bsm*I restriction enzyme (New England BioLabs) was used to digest the 290-bp PCR products. For *VEGFR2* 1192G/A polymorphism, *BstZ17I*-HF restriction enzyme (New England BioLabs) was used to digest the 262-bp PCR products. For *VEGFR2* 1719A/T polymorphism, *Alu*I restriction enzyme (New England BioLabs) was used to digest the 404-bp PCR products. For *VEGFR3* (rs72816988) polymorphism, the amplified products of 218 bp were digested with *Aci*I restriction enzyme (New England BioLabs). The digestion was done for overnight at 37 °C for *VEGFR1*-710C/T, *VEGFR2* 1192G/A, *VEGFR2* 1719A/T and *VEGFR3* (rs72816988) polymorphisms, whereas for *VEGFR2*-604 T/C polymorphism digestion was done at 65 °C. Restriction digestion products were analyzed on 2.4% agarose gel. Genotype was assigned to each sample on the basis of fragments obtained after digestion. The amplification and genotype conditions are given in Table 3. Randomly 10% samples were repeated by Sanger sequencing to revalidate the results and 100% concordance was found (Figs. 2, 3, 4, 5, 6).

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was evaluated to compare the observed and expected genotype frequencies among controls using Chi-square test. The differences in the genotype and allele frequencies of *VEGFR1*, *VEGFR2* and *VEGFR3* polymorphisms between the patients and controls were compared. Odds ratio (OR) and 95% confidence intervals were calculated by MedCalc software [32] to find the association of alleles and genotypes with esophageal cancer risk. SNPstats online software was used to study the different genetic models and haplotypes [33]. *p*-value less than 0.05 was considered as statistically significant for all the statistical analysis.

Results

A total of 310 esophageal cancer patients and 325 healthy controls were analyzed in this study. The genotype distribution for the studied *VEGFR2* polymorphisms was in Hardy–Weinberg equilibrium in controls.

Association of VEGFR1-710C/T polymorphism

The frequency of the CC and CT genotype of *VEGFR1*-710C/T polymorphism was 98.39 vs 94.46% and 1.61 vs 5.54% in patients and controls, respectively (Table 4). TT genotype was not observed in any of the subjects. CT genotype (OR = 0.28; 95%CI, 0.10–0.76; $p = 0.01$) and T allele (OR = 0.28; 95%CI, 0.10–0.77; $p = 0.01$) was found to be significantly associated with decreased risk of esophageal cancer.

Association of VEGFR2 polymorphisms

The frequency of TC genotype of *VEGFR2*-604 T/C polymorphism was higher in controls (Table 4) and was associated with decreased risk of esophageal cancer (OR = 0.66; 95%CI, 0.44–0.97; $p = 0.03$). After stratification of the data according to gender, TC genotype of *VEGFR2*-604 T/C was found to be significantly associated with decreased risk of esophageal cancer in male group (OR = 0.50; 95%CI, 0.28–0.89; $p = 0.02$) (Table 5). Genetic model analysis of *VEGFR2*-604 T/C

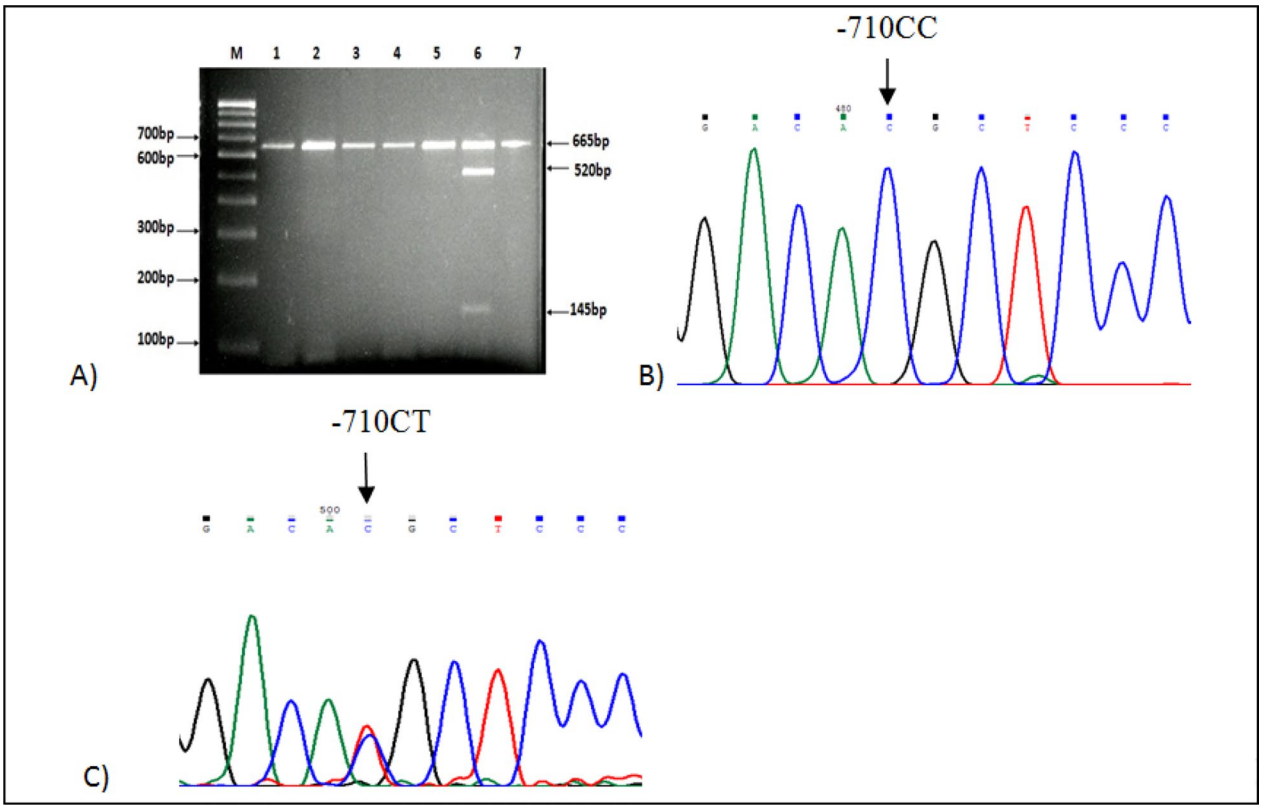


Fig. 2 **A** Photograph of 2.4% agarose gel showing the digested products. **B** Sequencing electropherogram representing CC genotype. **C** CT genotype of *VEGFR1*-710C/T polymorphism

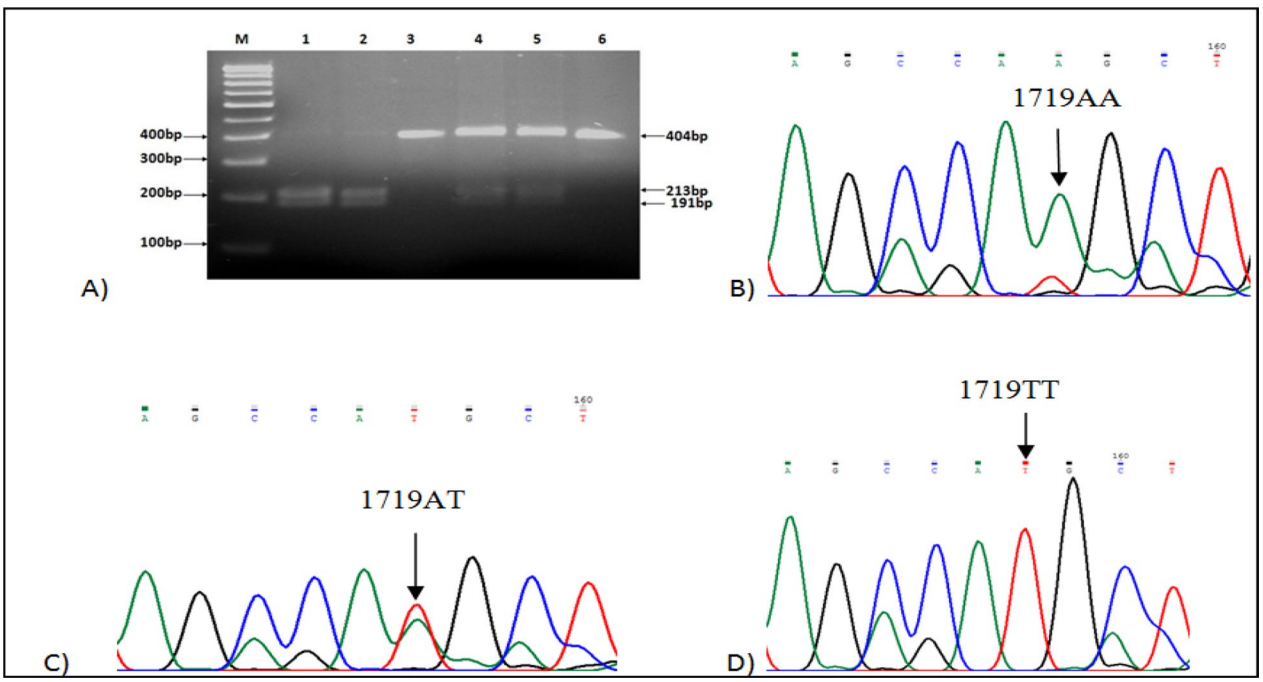


Fig. 3 **A** Photograph of 2.4% agarose gel showing the digested products. **B** Sequencing electropherogram representing AA genotype. **C** AT genotype and **D** TT genotype of *VEGFR2* 1719A/T polymorphism

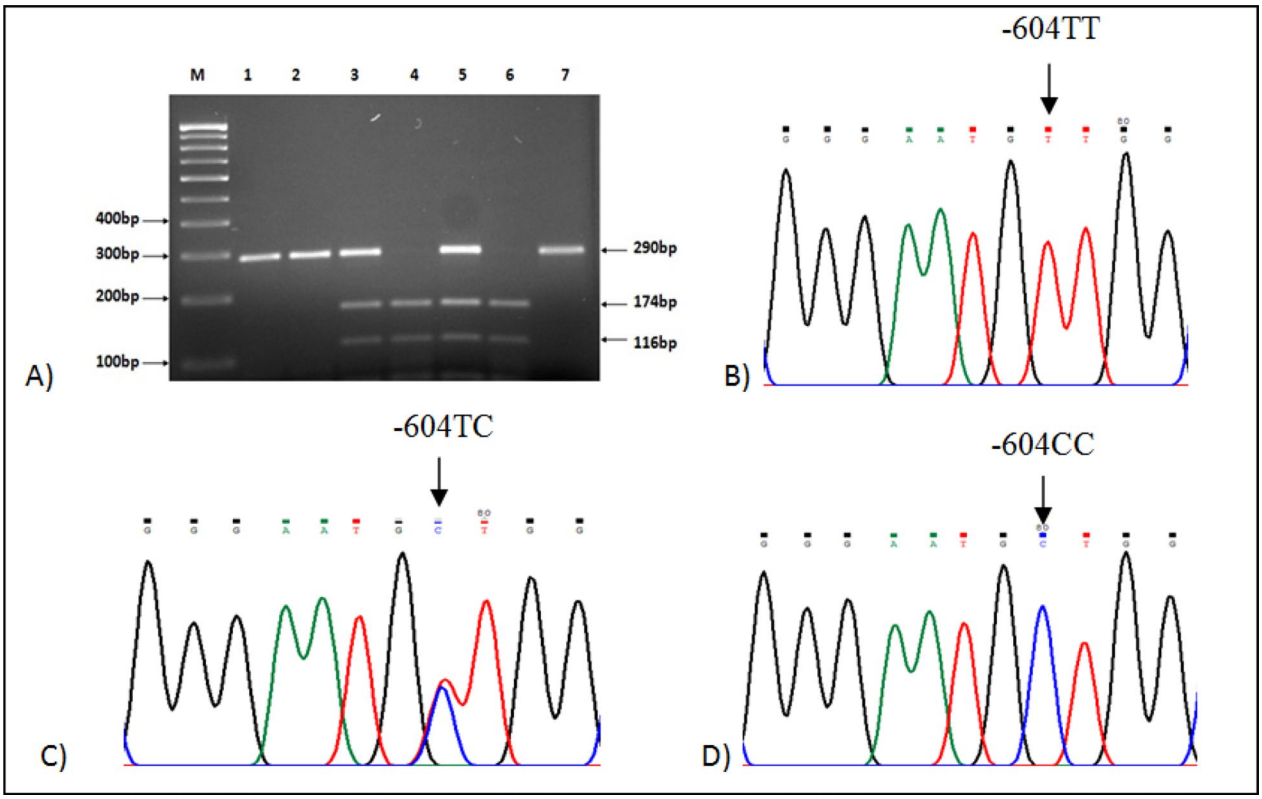


Fig. 4 **A** Photograph of 2.4% agarose gel showing the digested products. **B** Sequencing electropherogram representing TT genotype. **C** TC genotype and **D** CC genotype of *VEGFR2*-604 T/C polymorphism

polymorphism showed a decreased risk of esophageal cancer under codominant (OR = 0.66; 95%CI, 0.44–0.97; $p = 0.03$) and dominant model (OR = 0.66; 95%CI, 0.46–0.96; $p = 0.03$) (Table 6). In male group, decreased esophageal cancer risk was observed under codominant (OR = 0.50; 95%CI, 0.28–0.89; $p = 0.02$) and dominant model (OR = 0.53; 95%CI, 0.31–0.92; $p = 0.02$) (Table 7).

In female group, GA genotype of *VEGFR2* 1192G/A polymorphism was found to be significantly associated with decreased esophageal cancer risk (OR = 0.54; 95%CI, 0.31–0.95; $p = 0.03$). Genetic model analysis of *VEGFR2* 1192G/A polymorphism revealed a significantly decreased esophageal cancer risk in codominant (OR = 0.54; 95%CI, 0.31–0.95; $p = 0.03$), dominant (OR = 0.56; 95%CI, 0.32–0.96; $p = 0.04$), and overdominant model (OR = 0.54; 95%CI, 0.31–0.95; $p = 0.03$) in female group. We further compare the genotype distribution of *VEGFR2* 1192G/A polymorphism between male patients and female patients and observed that GA genotype was significantly associated with increased risk of esophageal cancer in male patients as compared to female patients (Table 8). There was no significant difference in the genotype and allele frequencies of *VEGFR2* 1719A/T polymorphism between patients and controls (Table 4).

Association of VEGFR3 (rs72816988) polymorphism

The frequency of the GG and GA genotype of *VEGFR3* (rs72816988) polymorphism was 95.16 vs 94.46% and 4.84 vs 5.54% in patients and controls, respectively. AA genotype was not observed in any of the subjects. There was no significant difference in genotype and allele frequencies between patients and controls (Table 4).

Haplotype analysis

To evaluate the combined effect of *VEGFR2* polymorphisms in the susceptibility to esophageal cancer, haplotype analysis was performed. In total subjects, haplotype $C\text{-}_{604}$ $A_{1719}A_{1192}$ was significantly associated with the decreased esophageal cancer risk (OR = 0.44; 95%CI,

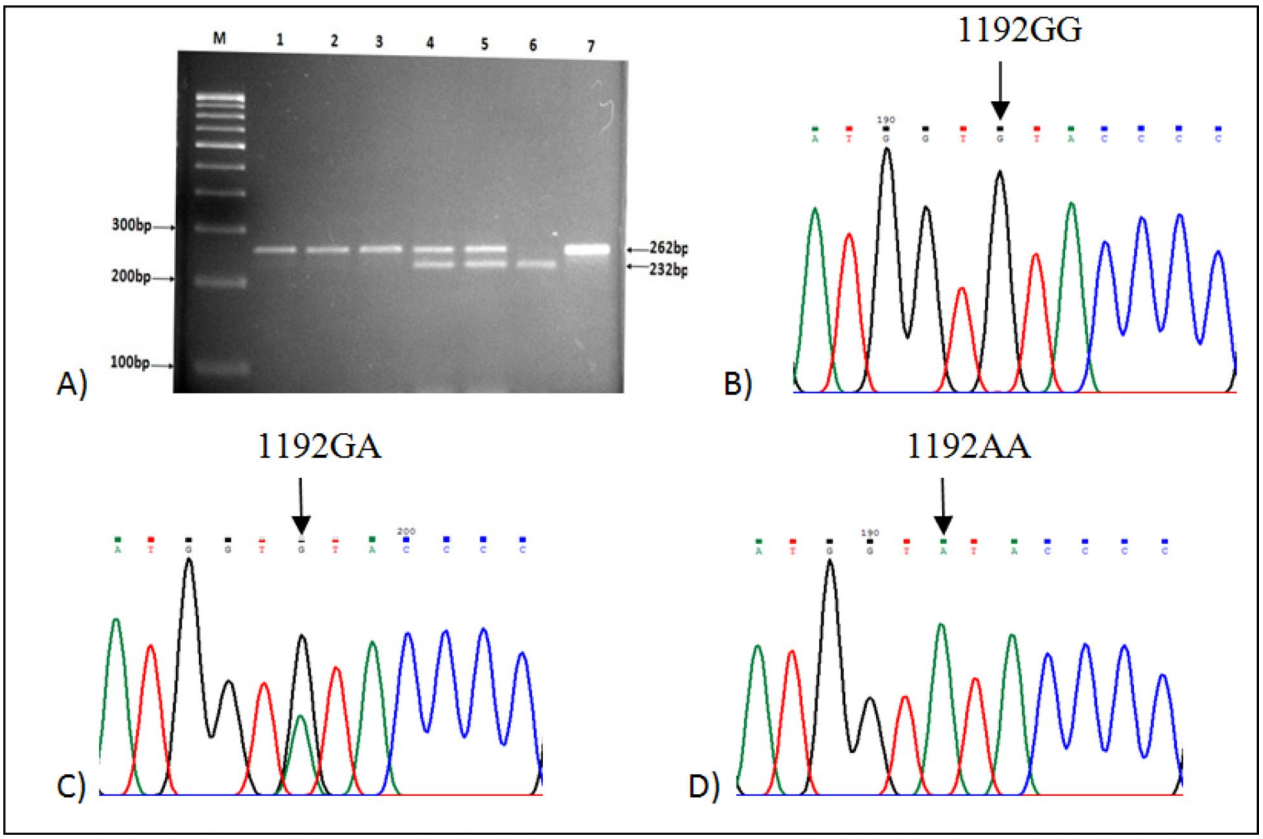


Fig. 5 **A** Photograph of 2.4% agarose gel showing the digested products. **B** Sequencing electropherogram representing GG genotype. **C** GA genotype and **D** AA genotype of *VEGFR2* 1192G/A polymorphism

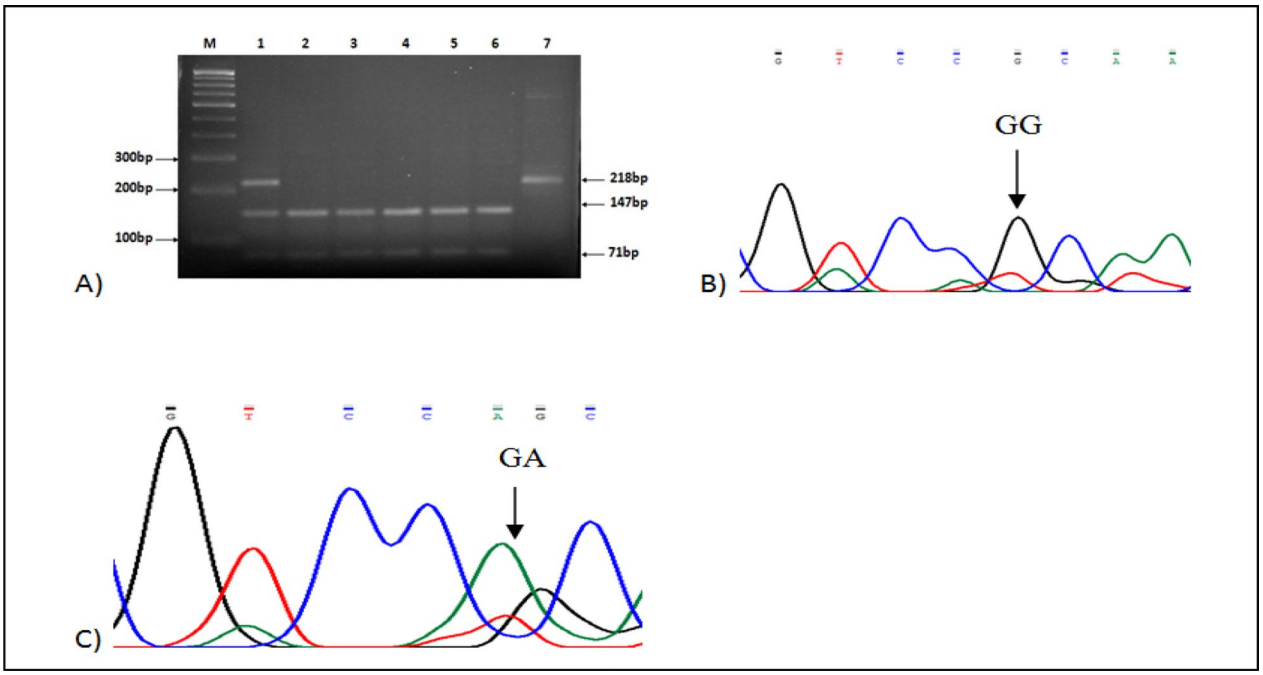


Fig. 6 **A** Photograph of 2.4% agarose gel showing the digested products. **B** Sequencing electropherogram representing GG genotype and **C** GA genotype of *VEGFR3* (rs72816988) polymorphism

Table 3 Details of analyzed *VEGFR* polymorphisms and genotyping conditions

Polymorphism (Chromosome location)	Location	Annealing temperature	Restriction enzyme used	Incubation temperature (°C)	Fragment size (bp)
VEGFR1-710 C/T (13q12.3)	Promoter	65 °C	*Nla*III	37	C allele-665 T allele-518,147
VEGFR2-604 T/C rs2071559 (4q12)	Promoter	62 °C	*Bsm*I	65	T allele-290 C allele-174,116
VEGFR2 1192G/A rs2305948 (4q12)	Exon 7	62 °C	*BstZ17I*-HF	37	G allele-262 A allele-232,30
VEGFR2 1719A/T rs1870377 (4q12)	Exon 11	58 °C	*Alu*I	37	T allele-404 A allele-213,191
VEGFR3 rs72816988 (5q35.3)	3′ UTR	60 °C	*Aci*I	37	A allele-218 G allele-147,71

Table 4 Comparison of genotype and allele frequency of *VEGFR* polymorphisms between esophageal cancer patients and controls

Polymorphism	Genotype/Allele	Patients *n* (%)	Controls *n* (%)	OR (95% CI)	*p* value
VEGFR1-710C/T	CC	305 (98.39)	307 (94.46)	Reference	
	CT	5 (1.61)	18 (5.54)	0.28 (0.10–0.76)	**0.01**
	TT	–	–	–	–
	C	615 (99.19)	632 (97.23)	Reference	
	T	5 (0.81)	18 (2.77)	0.28 (0.10–0.77)	**0.01**
VEGFR2-604 T/C (rs2071559) HWE (*p* value) Patients: 0.36 Controls: 0.29	TT	85 (27.42)	65 (20.00)	Reference	
	TC	147 (47.42)	171 (52.61)	0.66 (0.44–0.97)	**0.03**
	CC	78 (25.16)	89 (27.39)	0.67 (0.43–1.04)	0.08
	T	317 (51.13)	301 (46.31)	Reference	
	C	303 (48.87)	349 (53.69)	0.82 (0.66–1.03)	0.08
VEGFR2 1192 G/A (rs2305948) HWE (*p* value) Patients: 0.81 Controls: 0.72	GG	248 (80.00)	248 (76.31)	Reference	
	GA	59 (19.03)	71 (21.85)	0.83 (0.56–1.22)	0.35
	AA	3 (0.97)	6 (1.84)	NC	NC
	G	555 (89.52)	567 (87.23)	Reference	
	A	65 (10.48)	83 (12.77)	0.80 (0.57–1.13)	0.20
VEGFR2 1719A/T (rs1870377) HWE (*p* value) Patients: 0.70 Controls: 0.50	AA	187 (60.32)	202 (62.15)	Reference	
	AT	106 (34.19)	111 (34.15)	1.03 (0.74–1.44)	0.85
	TT	17 (5.49)	12 (3.70)	1.53 (0.71–3.29)	0.27
	A	480 (77.42)	515 (79.23)	Reference	
	T	140 (22.58)	135 (20.77)	1.11 (0.85–1.45)	0.43
VEGFR3 (rs72816988)	GG	295 (95.16)	307 (94.46)	Reference	
	GA	15 (4.84)	18 (5.54)	0.87 (0.43–1.75)	0.69
	AA	–	–	–	–
	G	605 (97.58)	632 (97.23)	Reference	
	A	15 (2.42)	18 (2.77)	0.87 (0.43–1.74)	0.69

OR odds ratio; *CI* confidence interval, *NC* not calculated (genotype count less than 5 has been excluded from analysis); statistically significant *p* values are presented in bold

0.23–0.84; $p=0.01$), whereas haplotype $C\text{-}_{604}$ $A_{1719}G_{1192}$ was marginally associated with the decreased cancer risk (OR = 0.74; 95%CI, 0.54–1.01; $p=0.06$). Haplotype $C\text{-}_{604}$ $A_{1719}G_{1192}$ was significantly associated with the decreased esophageal cancer risk in male group (OR = 0.48; 95%CI, 0.28–0.80; $p=0.006$) (Table 9).

Table 5 Analysis of *VEGFR* polymorphisms and esophageal cancer risk in male and female subjects

Polymorphism	Genotype/ Allele	Male (Patients: 137; Controls: 144)				Female (Patients: 173; Controls: 181)			
		Patients *n* (%)	Controls *n* (%)	OR (95% CI)	*p* value	Patients *n* (%)	Controls *n* (%)	OR (95% CI)	*p* value
VEGFR1-710C/T	CC	135 (98.54)	137 (95.14)	Reference		170 (98.26)	170 (93.92)	Reference	
	CT	2 (1.46)	7 (4.86)	NC	NC	3 (1.74)	11 (6.08)	NC	NC
	TT	0	0	NC	NC	0	0	NC	NC
	C	272 (99.27)	281 (97.57)	Reference		343 (99.13)	351 (96.96)	Reference	
	T	2 (0.73)	7 (2.43)	NC	NC	3 (0.87)	11 (3.04)	NC	NC
VEGFR2-604 T/C	TT	44 (32.12)	29 (20.14)	Reference		41 (23.70)	36 (19.89)	Reference	
	TC	60 (43.80)	79 (54.86)	0.50 (0.28–0.89)	**0.02**	87 (50.29)	92 (50.83)	0.83 (0.49–1.42)	0.49
	CC	33 (24.08)	36 (25.00)	0.60 (0.31–1.17)	0.14	45 (26.01)	53 (29.28)	0.74 (0.41–1.36)	0.34
	T	148 (54.01)	137 (47.57)	Reference		169 (48.84)	164 (45.30)	Reference	
	C	126 (45.99)	151 (54.43)	0.77 (0.55–1.08)	0.13	177 (51.16)	198 (54.70)	0.87 (0.64–1.16)	0.34
VEGFR2 1192 G/A	GG	100 (72.99)	109 (75.70)	Reference		148 (85.55)	139 (76.79)	Reference	
	GA	36 (26.28)	31 (21.53)	1.26 (0.73–2.19)	0.40	23 (13.29)	40 (22.10)	0.54 (0.31–0.95)	**0.03**
	AA	1 (0.73)	4 (2.77)	NC	NC	2 (1.16)	2 (1.11)	NC	NC
	G	236 (86.13)	249 (86.46)	Reference		319 (92.20)	318 (87.84)	Reference	
	A	38 (13.87)	39 (13.54)	1.03 (0.63–1.66)	0.91	27 (7.80)	44 (12.16)	0.61 (0.37–1.01)	**0.05**
VEGFR2 1719A/T	AA	76 (55.47)	95 (65.97)	Reference		111 (64.16)	107 (59.12)	Reference	
	AT	55 (40.15)	45 (31.25)	1.53 (0.93–2.51)	0.09	51 (29.48)	66 (36.46)	0.74 (0.47–1.17)	0.20
	TT	6 (4.38)	4 (2.78)	NC	NC	11 (6.36)	8 (4.42)	1.32 (0.51–3.42)	0.56
	A	207 (75.55)	235 (81.60)	Reference		273 (78.90)	280 (77.35)	Reference	
	T	67 (24.45)	53 (18.40)	1.43 (0.96–2.15)	0.08	73 (21.30)	82 (22.65)	0.91 (0.64–1.30)	0.62
VEGFR3 rs72816988	GG	131 (95.62)	135 (93.75)	Reference		164 (94.80)	172 (95.03)	Reference	
	GA	6 (4.38)	9 (6.25)	0.69 (0.24–1.98)	0.49	9 (5.20)	9 (4.97)	1.05 (0.41–2.71)	0.92
	AA	0	0	–	–				
	G	268 (97.81)	279 (96.87)	Reference		337 (97.40)	353 (97.51)	Reference	
	A	6 (2.19)	9 (3.13)	0.69 (0.24–1.98)	0.49	9 (2.60)	9 (2.49)	1.05 (0.41–2.67)	0.92

OR odds ratio; *CI* confidence interval, *NC* not calculated (genotype count less than 5 has been excluded from analysis); statistically significant *p* values are presented in bold

Discussion

In the present study, we have investigated the association of *VEGFR1*-710C/T, *VEGFR2*-604 T/C, *VEGFR2* 1192 G/A, *VEGFR2* 1719A/T and *VEGFR3* (rs72816988) polymorphisms with esophageal cancer risk. Researchers have investigated these polymorphisms in different cancers and results are variable (Supplementary Tables 1–4). So far, the role of these polymorphisms has not been explored in esophageal cancer. In the present study, T allele of *VEGFR1*-710C/T polymorphism was significantly associated with decreased risk of esophageal cancer. Till date, there is no published study on *VEGFR1*-710C/T polymorphism in gastrointestinal tract cancer. Association of T allele with reduced breast cancer risk has been reported in Spanish population [29], whereas no association of *VEGFR1*-710C/T polymorphism with breast cancer risk has been reported in patients from Punjab North-west India [34].

The promoter polymorphism *VEGFR2*-604 T/C changes the binding site for transcription factor E2F in *KDR* promoter region, which can downregulate KDR expression [14]. In the present study, TC genotype of *VEGFR2*-604 T/C polymorphism was significantly associated with reduced risk of esophageal cancer. The TC + CC combined genotype of *VEGFR2*-604 T/C polymorphism was associated with decreased esophageal cancer risk in dominant model. Contrary to our results, combined TC + CC genotype was significantly associated with increased risk of colorectal cancer in Korean population [35]. The C allele of *VEGFR2*-604 T/C polymorphism was associated with increased risk of pancreatic cancer in Romanian population [15]. However, no correlation of *VEGFR2*-604 T/C polymorphism has been observed with gastric cancer [19] and hepatocellular carcinoma in Chinese population [17]. In Danish population, TT + TC genotype of *VEGFR2*-604 T/C was associated with improved overall survival in colorectal cancer patients [16].

VEGFR2 1192G/A polymorphism located in third NH2 terminal Ig-like domains in the extracellular region

Table 6 Relationship between genetic models of *VEGFR2* polymorphisms and the risk of esophageal cancer

Polymorphism	Model	Genotype	Patients *n* (%)	Control *n* (%)	OR (95% CI)	*p* value
VEGFR2-604 T/C	Codominant	TT	85 (27.42)	65 (20.00)	Reference	
		TC	147 (47.42)	171 (52.61)	0.66 (0.44–0.97)	**0.03**
		CC	78 (25.16)	89 (27.39)	0.67 (0.43–1.04)	0.08
	Dominant	TT	85 (27.42)	65 (20.00)	Reference	
		TC + CC	225 (72.58)	260 (80.00)	0.66 (0.46–0.96)	**0.03**
	Recessive	TT + TC	232 (74.84)	236 (72.62)	Reference	
		CC	78 (25.16)	89 (27.38)	0.89 (0.62–1.27)	0.52
	Overdominant	TT + CC	163 (52.58)	154 (47.38)	Reference	
		TC	147 (47.42)	171 (52.62)	0.81 (0.59–1.11)	0.19
VEGFR2 1719A/T	Codominant	AA	187 (60.32)	202 (62.15)	Reference	
		AT	106 (34.19)	111 (34.15)	1.03 (0.74–1.44)	0.85
		TT	17 (5.49)	12 (3.70)	1.53 (0.71–3.29)	0.27
	Dominant	AA	187 (60.32)	202 (62.15)	Reference	
		AT + TT	123 (39.68)	123 (37.85)	1.08 (0.78–1.49)	0.63
	Recessive	AA + AT	293 (94.52)	313 (96.31)	Reference	
		TT	17 (5.48)	12 (3.69)	1.51 (0.71–3.22)	0.28
	Overdominant	AA + TT	204 (65.81)	214 (65.85)	Reference	
		AT	106 (34.19)	111 (34.15)	1.00 (0.72–1.39)	0.99
VEGFR2 1192G/A	Codominant	GG	248 (80.00)	248 (76.31)	Reference	
		GA	59 (19.03)	71 (21.85)	0.83 (0.56–1.22)	0.35
		AA	3 (0.97)	6 (1.84)	NC	NC
	Dominant	GG	248 (80.00)	248 (76.31)	Reference	
		GA + AA	62 (20.00)	77 (23.69)	0.81 (0.55–1.17)	0.26
	Recessive	GG + GA	307 (99.03)	319 (98.15)	Reference	
		AA	3 (0.97)	6 (1.85)	NC	NC
	Overdominant	GG + AA	251 (80.97)	254 (78.15)	Reference	
		GA	59 (19.03)	71 (21.85)	0.84 (0.57–1.24)	0.38

OR odds ratio; *CI* confidence interval, statistically significant *p* values are presented in bold, *NC* not calculated (genotype count less than 5 has been excluded from analysis)

is crucial for ligand binding [14]. In the present study, no association was found between *VEGFR2* 1192G/A polymorphism and esophageal cancer in total subjects. However, combined GA + AA genotype was significantly associated with decreased esophageal cancer risk in female group. Association of combined GA + AA genotype of *VEGFR2* 1192G/A polymorphism with increased colorectal cancer risk has been documented in Korean population [35]. In Danish population, GG genotype of *VEGFR2* 1192G/A polymorphism was associated with improved survival in patients having colorectal cancer [16]. GA genotype was associated with lower overall survival in hepatocellular cancer Han Chinese patients [17]. No correlation was observed between *VEGFR2* 1192G/A polymorphism and gastric cancer in Chinese population [19].

In the present study, *VEGFR2* 1719A/T polymorphism was not associated with esophageal cancer risk. *VEGFR2* 1719A/T polymorphism was not associated with recurrence and overall survival in esophageal adenocarcinoma patients who underwent surgery [36]. Similarly, no association of *VEGFR2* 1719A/T polymorphism has been reported in hepatocellular carcinoma in Han Chinese [17] and colorectal cancer in Danish patients [16]. The T allele of *VEGFR2* 1719A/T polymorphism was associated with increased hepatocellular cancer risk in Portuguese patients [18], whereas AA genotype was associated with poor prognosis in Chinese gastric cancer patients [19].

No significant association was observed between *VEGFR3* (rs72816988) polymorphism and esophageal cancer risk in the present study. Till date, there is no published study on *VEGFR3* (rs72816988) polymorphism in GIT cancer. Relationship between *VEGFR3* (rs72816988) polymorphism with the clinical outcomes of renal cell carcinoma patients treated with sorafenib [37] and sunitinib [31] has been studied, and no association was found in both of these studies.

Table 7 Genetic model analysis of *VEGFR2* polymorphisms with esophageal cancer risk in male and female subjects

Polymorphism	Model	Male					Female			
		Genotype	Patients *n* (%)	Controls *n* (%)	OR (95%CI)	*p* value	Patients *n* (%)	Controls *n* (%)	OR (95%CI)	*p* value
VEGFR2-604 T/C	Codominant	TT	44 (32.12)	29 (20.14)	Reference		41 (23.70)	36 (19.89)	Reference	
		TC	60 (43.80)	79 (54.86)	0.50 (0.28–0.89)	**0.02**	87 (50.29)	92 (50.83)	0.83 (0.49–1.42)	0.49
		CC	33 (24.08)	36 (25.00)	0.60 (0.31–1.17)	0.14	45 (26.01)	53 (29.28)	0.74 (0.41–1.36)	0.34
	Dominant	TT	44 (32.12)	29 (20.14)	Reference		41 (23.70)	36 (19.89)	Reference	
		TC + CC	93 (67.88)	115 (79.86)	0.53 (0.31–0.92)	**0.02**	132 (76.30)	145 (80.11)	0.79 (0.48–1.32)	0.38
	Recessive	TT + TC	104 (75.91)	108 (75.00)	Reference		128 (73.99)	128 (70.72)	Reference	
		CC	33 (24.09)	36 (25.00)	0.95 (0.55–1.64)	0.86	45 (26.01)	53 (29.28)	0.85 (0.53–1.35)	0.49
	Overdominant	TT + CC	77 (56.20)	65 (45.14)	Reference		86 (49.71)	89 (49.17)	Reference	
		TC	60 (43.80)	79 (54.86)	0.64 (0.40–1.03)	0.06	87 (50.29)	92 (50.83)	0.98 (0.64–1.48)	0.92
VEGFR2 1719A/T	Codominant	AA	76 (55.47)	95 (65.97)	Reference		111 (64.16)	107 (59.12)	Reference	
		AT	55 (40.15)	45 (31.25)	1.53 (0.93–2.51)	0.09	51 (29.48)	66 (36.46)	0.74 (0.47–1.17)	0.20
		TT	6 (4.38)	4 (2.78)	NC	NC	11 (6.36)	8 (4.42)	1.32 (0.51–3.42)	0.56
	Dominant	AA	76 (55.47)	95 (65.97)	Reference		111 (64.16)	107 (59.12)	Reference	
		AT + TT	61 (44.53)	49 (34.03)	1.56 (0.96–2.52)	0.07	62 (35.84)	74 (40.88)	0.81 (0.52–1.24)	0.33
	Recessive	AA + AT	131 (95.62)	140 (97.22)	NC	NC	162 (93.64)	173 (95.58)	Reference	
		TT	6 (4.38)	4 (2.78)			11 (6.36)	8 (4.42)	1.47 (0.58–3.74)	0.42
	Overdominant	AA + TT	82 (59.85)	99 (68.75)	Reference		122 (70.52)	115 (63.54)	Reference	
		AT	55 (40.15)	45 (31.25)	1.48 (0.90–2.41)	0.12	51 (29.48)	66 (36.46)	0.73 (0.47–1.14)	0.16
VEGFR2 1192G/A	Codominant	GG	100 (72.99)	109 (75.70)	Reference		148 (85.55)	139 (76.79)	Reference	
		GA	36 (26.28)	31 (21.53)	1.26 (0.73–2.19)	0.40	23 (13.29)	40 (22.10)	0.54 (0.31–0.95)	**0.03**
		AA	1 (0.73)	4 (2.77)	NC	NC	2 (1.16)	2 (1.11)	NC	NC
	Dominant	GG	100 (72.99)	109 (75.69)	Reference		148 (85.55)	139 (76.80)	Reference	
		GA + AA	37 (27.01)	35 (24.31)	1.15 (0.67–1.97)	0.60	25 (14.45)	42 (23.20)	0.56 (0.32–0.96)	**0.04**
	Recessive	GG + GA	136 (99.27)	140 (97.22)	NC	NC	171 (98.84)	179 (98.89)	Reference	
		AA	1 (0.73)	4 (2.78)			2 (1.16)	2 (1.11)	NC	NC
	Overdominant	GG + AA	101 (73.72)	113 (78.47)	Reference		150 (86.70)	141 (77.90)	Reference	
		GA	36 (26.28)	31 (21.53)	1.29 (0.75–2.25)	0.35	23 (13.30)	40 (22.10)	0.54 (0.31–0.95)	**0.03**

OR odds ratio; *CI* confidence interval, *NC* not calculated (genotype count less than 5 has been excluded from analysis); statistically significant p values are presented in bold

Table 8 Association of *VEGFR* polymorphisms with esophageal cancer risk in male and female patients

Polymorphism	Genotype/Allele	Males *n* (%)	Females *n* (%)	OR (95% CI)	*p* value
VEGFR1-710C/T	CC	135 (98.54)	170 (98.26)	Reference	
	CT	2 (1.46)	3 (1.74)	–	–
	TT	0 (0)	0 (0)	–	–
	C	272 (99.27)	343 (99.13)	Reference	
	T	2 (0.73)	3 (0.87)	NC	NC
VEGFR2-604 T/C (rs2071559)	TT	44 (32.12)	41 (23.70)	Reference	
	TC	60 (43.80)	87 (50.29)	0.64 (0.37–1.10)	0.11
	CC	33 (24.08)	45 (26.01)	0.68 (0.37–1.27)	0.23
	T	148 (54.01)	169 (48.84)	Reference	
	C	126 (45.99)	177 (51.16)	0.81 (0.59–1.12)	0.20
VEGFR2 1192 G/A (rs2305948)	GG	100 (72.99)	148 (85.55)	Reference	
	GA	36 (26.28)	23 (13.29)	2.32 (1.29–4.14)	**0.005**
	AA	1 (0.73)	2 (1.16)	NC	NC
	G	236 (86.13)	319 (92.20)	Reference	
	A	38 (13.87)	27 (7.80)	1.90 (1.13–3.20)	**0.01**
VEGFR2 1719A/T (rs1870377)	AA	76 (55.47)	111 (64.16)	Reference	
	AT	55 (40.15)	51 (29.48)	1.57 (0.97–2.54)	0.06
	TT	6 (4.38)	11 (6.36)	0.79 (0.28–2.25)	0.67
	A	207 (75.55)	273 (78.90)	Reference	
	T	67 (24.45)	73 (21.10)	1.21 (0.83–1.76)	0.32
VEGFR3 (rs72816988)	GG	131 (95.62)	164 (94.80)	Reference	
	GA	6 (4.38)	9 (5.20)	0.83 (0.29–2.40)	0.74
	AA	0 (0)	0 (0)	NC	NC
	G	268 (97.81)	337 (97.40)	Reference	
	A	6 (2.18)	9 (2.60)	0.84 (0.29–2.38)	0.74

OR = odds ratio; CI = confidence interval, NC = not calculated (genotype count less than 5 has been excluded from analysis); statistically significant p values are presented in bold

In the present study, C-$_{604}$ A$_{1719}$A$_{1192}$ haplotype of *VEGFR2* was significantly associated with decreased esophageal cancer risk in total subjects, whereas C-$_{604}$ A$_{1719}$G$_{1192}$ haplotype was associated with decreased esophageal cancer risk in male group. Association of C-$_{604}$G$_{1192}$ and C-$_{604}$A$_{1192}$ haplotypes with an increased risk to colorectal cancer has been reported in Korean patients [35]. The response of *VEGFR* polymorphisms with different therapies in GIT cancers has been studied in different populations and reported association with disease survival (Supplementary Tables 5–7).

Strength of the study

So far, the present case–control study is the first study which have analyzed the association of *VEGFR1*-710C/T, *VEGFR2*-604 T/C, *VEGFR2* 1192 G/A, *VEGFR2* 1719A/T and *VEGFR3* (rs72816988) polymorphisms with esophageal cancer risk. It provides the baseline data for genetic polymorphisms of angiogenic pathway.

Limitations of the study

The present study only focuses on the population of Punjab, North-west India, with a limited sample size; however, the frequency of genetic polymorphisms often varies between different ethnic groups.

Conclusion and future directions

In the present study, we found that *VEGFR1*-710C/T, *VEGFR2*-604 T/C and *VEGFR2* 1192G/A polymorphisms were associated with decreased risk of esophageal cancer in the patients from Punjab, North-west India. In future, further studies with larger sample size on different ethnic groups are required to better understand the role of *VEGFR* polymorphisms in the development and progression of esophageal cancer. Understanding the relationship between *VEGFR* polymorphisms and esophageal cancer risk can aid in identifying individuals at higher risk and facilitate early detection and intervention

Table 9 *VEGFR2* polymorphism haplotypes and esophageal cancer risk

Haplotype	Patients' frequency	Controls' frequency	OR(95%CI)	*p* value
Total				
T_{-604} A_{1719} G_{1192}	0.39	0.32	Reference	
C_{-604} $A_{1719}G_{1192}$	0.32	0.37	0.74 (0.54–1.01)	0.06
C_{-604} $T_{1719}G_{1192}$	0.11	0.09	0.98 (0.63–1.54)	0.37
T_{-604} $T_{1719}G_{1192}$	0.08	0.09	0.69 (0.40–1.20)	0.19
C_{-604} $A_{1719}A_{1192}$	0.04	0.07	0.44 (0.23–0.84)	**0.01**
T_{-604} $A_{1719}A_{1192}$	0.03	0.03	0.62 (0.23–1.65)	0.34
Males				
T_{-604} A_{1719} G_{1192}	0.42	0.32	Reference	
C_{-604} $A_{1719}G_{1192}$	0.26	0.38	0.48 (0.28–0.80)	**0.006**
C_{-604} $T_{1719}G_{1192}$	0.10	0.06	1.45 (0.64–3.31)	0.37
T_{-604} $T_{1719}G_{1192}$	0.08	0.11	0.51 (0.23–1.16)	0.11
C_{-604} $A_{1719}A_{1192}$	0.04	0.07	0.52 (0.21–1.29)	0.16
T_{-604} $A_{1719}A_{1192}$	0.03	0.05	0.37 (0.10–1.40)	0.15
Females				
C_{-604} $A_{1719}G_{1192}$	0.36	0.35	Reference	
T_{-604} A_{1719} G_{1192}	0.37	0.32	1.14 (0.76–1.69)	0.52
C_{-604} $T_{1719}G_{1192}$	0.11	0.11	1.02 (0.56–1.84)	0.95
T_{-604} $T_{1719}G_{1192}$	0.08	0.09	0.87 (0.46–1.67)	0.68
C_{-604} $A_{1719}A_{1192}$	0.04	0.07	0.57 (0.25–1.28)	0.17
T_{-604} $A_{1719}A_{1192}$	0.02	0.02	0.79 (0.19–3.33)	0.64

OR odds ratio; *CI* confidence interval, statistically significant *p* values are displayed in bold

which is crucial for better prognosis. In future, studies examining the relationship between *VEGFR* polymorphisms and the response of esophageal cancer patients to chemotherapeutic drugs are required. This will help to understand how these polymorphisms affect treatment response and aid in the provision of personalized medicine, which aims to maximize therapeutic outcomes and minimizing adverse effects.

Abbreviations

VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
GIT	Gastrointestinal tract
SNP	Single-nucleotide polymorphism
PCR–RFLP	Polymerase chain reaction–restriction fragment length polymorphism
EDTA	Ethylenediaminetetraacetic acid
HWE	Hardy–Weinberg equilibrium
OR	Odds ratio
CI	Confidence interval

Supplementary Information

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Additional file 1.

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Author contributions
KG and VS designed the study. SKW performed the experiments and analyzed the data. SKW and KG prepared the manuscript. MSU and MS did clinical diagnosis of patients and also helped in collection of blood samples of patients. All authors read and approved the final manuscript.

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Availability of data and materials
All the data relevant to this study has been included in the manuscript or uploaded as supplementary files.

Declarations

Ethics approval and consent to participate
This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Guru Nanak Dev University, Amritsar, Punjab (India). Informed consent was obtained from all individual participants included in the present study.

Competing interests
All the authors declare that they have no conflicts of interest.

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