REVIEW

Editorial Process: Submission:05/23/2023 Acceptance:09/15/2023

Association of *VEGF*-116G/A Promoter Polymorphism with Esophageal Cancer Risk: A Case-Control study and an Updated Meta-Analysis on Gastrointestinal Tract Cancers

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Abstract

Objective: The present study aimed to investigate the potential association of *VEGF*-116G/A promoter polymorphism with esophageal cancer risk in North-West Indians and to perform a comprehensive meta-analysis of *VEGF*-116G/A polymorphism in Gastrointestinal Tract (GIT) cancers. **Methods:** A total of 679 DNA samples (333 esophageal cancer patients and 346 healthy controls) were genotyped for *VEGF*-116G/A polymorphism using Sanger sequencing. In silico analysis was carried out to predict the impact of *VEGF*-116G/A polymorphism on transcription factor binding sites. Ten studies including 2157 patients and 2307 controls on different GIT cancers were included in the meta-analysis. **Results:** The AA genotype and A allele of *VEGF* -116G/A polymorphism was significantly associated with an increased risk of esophageal cancer. In silico analysis predicted that A allele of *VEGF*-116G/A polymorphism created new binding sites for STAT4, c-Ets-1 and Elk-1 transcription factors. The meta-analysis results showed that *VEGF*-116G/A polymorphism was associated with an increased risk of GIT cancer under the recessive and AA vs GG genetic model in the overall population. Stratification of the studies by ethnicity revealed an increased risk of GIT cancers in Asians under allele contrast, recessive, AA vs GG and AA vs AG model. Analysis based on cancer type revealed an increased risk of esophageal cancer under allele contrast, recessive, AA vs GG and AA vs AG comparison model and increased risk of oral cancer was observed under the allele contrast model and dominant model. **Conclusion:** *VEGF*-116G/A polymorphism was associated with esophageal cancer risk in North- West Indians. The findings of the present meta-analysis showed a significant association of *VEGF*-116G/A polymorphism with GIT cancer risk.

Keywords: *VEGF*- esophageal cancer- polymorphisms- meta-analysis

Asian Pac J Cancer Prev, **24 (9)**, 2951-2962

Introduction

Esophageal cancer (EC) ranks seventh among the most common cancers worldwide (Sung et al., 2021) and is a complex, rapidly growing malignancy with significant heterogeneity in ethnicity, incidence and aetiology. The incidence and mortality of EC are increasing at an alarming rate despite of many advancements in the diagnosis of premalignant lesions and the development of adjuvant and neoadjuvant therapies (Ferlay et al., 2015). EC has poor prognostic outcomes with no symptoms appearing in the early stages of the disease (Veugelers et al., 2006). The major hotspots of EC are in developing regions where esophageal squamous cell carcinoma (ESCC) is the most common histological type (Van loon et al., 2018). The major risk factors of ESCC include high alcohol consumption, use of nonconventional tobacco products, low socioeconomic status and poor oral hygiene (Mir and Dar, 2009).

Genetic variability plays an important role in the pathogenesis of esophageal cancer. Genome-wide association studies have identified many variants in genes involved in the folate metabolism pathway, energy pathway, cell growth and DNA repair pathway to be associated with an increased risk of EC (Chen et al., 2021).

Hypoxia is a major regulator of the "angiogenic switch" during tumour growth and development (Diaz-Gonzalez et al., 2005). *In vitro* and *in vivo* studies demonstrated that hypoxic conditions upregulate the levels of *VEGF* mRNA (Alvarez Arroyo et al., 2002). VEGF, a constitutively expressed glycoprotein plays a key role in regulating tumour-related angiogenesis (Carmeliet, 2005). VEGF is encoded by *VEGF*A, a highly polymorphic gene located at 6p21.3 (Vincenti et al., 1996). Several

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polymorphisms have been identified in the promoter, 5′ and 3′ Untranslated regions of *VEGF* and some of them were associated with alteration in *VEGF* expression in tissues (Brogan et al., 1999; Watson et al., 2000). One such functional polymorphism located within the promoter region is *VEGF*-116G/A or -1154G/A. Functional analysis revealed that the GG genotype of the *VEGF* -116G/A polymorphism was associated with increased *VEGF* production (Shahbazi et al., 2002). *VEGF*-116G/A polymorphism has been studied in several gastrointestinal cancers including oral (Kammerer et al., 2010; Supic et al., 2012), gastric (Tzanakis et al., 2006), colon (Cacev et al., 2008), colorectal (Yamamori et al., 2004; Ungerback et al., 2009; Dassoulas et al., 2009; Choi et al., 2011; Jang et al., 2013), gall bladder (Mishra et al., 2013) and hepatocellular cancer (Wu et al., 2009; Wu et al., 2013; Song et al., 2014; Baitello et al., 2016) and results were inconsistent.

Genetic polymorphisms in the angiogenesis-related genes are considered a potential prognostic marker for clinical outcomes as these are associated with tumour susceptibility, tumour recurrence, poor survival and response to anti-*VEGF* agents. *VEGF*-116A allele has been associated with acute toxicities such as cheilitis and leukopenia in Japanese esophageal squamous cell patients treated with 5-Fluorouracil/ Cisplatin/radiation (Sakaeda et al., 2008). The *VEGF*-116GG genotype was associated with worse overall survival in colorectal cancer patients treated with bevacizumab (Koutras et al., 2012). In Chinese hepatocellular carcinoma patients, *VEGF*-116AA genotype was associated with an increased risk of death and shorter survival time (Song et al., 2014). To date, no study has evaluated the association of *VEGF*-116G/A polymorphism and esophageal cancer among Indians. Gastrointestinal tract (GIT) cancers are the deadliest group of cancers affecting the digestive tract. GIT cancers account for 26% of cancer incidence and 35% of cancer-related deaths worldwide (Arnold et al., 2020). There are several risk factors for GIT cancers which include genetic, environmental and multi-causal combinations (Huang et al., 2021).There is a rapid turnover of epithelial cells in gastrointestinal tissues, thus leading to the accumulation of oncogenic mutations (Li et al., 2014). It has been reported that VEGF overexpression was associated with tumour progression and poor prognosis in several gastrointestinal tumours including esophageal carcinoma (Chen et al., 2012), gastric carcinoma (Maeda et al., 1996), pancreatic carcinoma (Costache et al., 2015) and oral cancer (Lin et al., 2016). Because of the functional significance of *VEGF*-116G/A polymorphism, several studies have evaluated the role of *VEGF*-116G/A polymorphism in different GIT cancers; however, the results were inconclusive. So far, three meta-analyses on *VEGF*-116G/A polymorphism have been conducted in GIT cancers including oral (Metzger et al., 2015), gastric (Zhuang et al., 2017) and colorectal (Zhou et al., 2011), which reported no association with the cancer risk. The aim of the present study was to evaluate the association of *VEGF* -116G/A polymorphism and esophageal cancer risk in North-West Indians and perform a comprehensive meta-analysis of *VEGF* -116G/A polymorphism in GIT cancer by including all the relevant articles on gastrointestinal cancer. To our knowledge this is the first study on *VEGF*-116G/A polymorphism in esophageal cancer risk and the present meta-analysis is the first meta-analysis investigating the association of *VEGF*-116G/A polymorphism with GIT cancer risk.

Materials and Methods

Case - control study

Selection of Subjects

The present case-control study analyzed a total of 679 subjects including 333 esophageal cancer patients (Mean age = 56.16±13.11 years) and 346 healthy controls (Mean age = 54.27±12.94 years). The study was approved by the institutional ethics committee of Guru Nanak Dev University, Amritsar and written informed consent was taken from all the study participants. The patients included in this study were clinically confirmed at Sri Guru Ram Das Institute of Medical Sciences, Amritsar. Esophageal cancer patients who were on treatment like chemotherapy, radiotherapy, surgery or had blood transfusion were not included in the present study. The control group consisted of randomly selected unrelated healthy individuals from the same geographical area as of EC patients and was matched by age and gender. The individuals with family history of cancer or any other chronic disease and on regular medications were not included in the control group. The personal history and disease history of each subject was recorded on a pre-structured proforma. There was higher frequency of smokers in the patient group (13.81%) than the control group (2.89%) More alcohol consumers in patients were reported compared to the control group (26.43% vs 19.36% respectively). The squamous cell carcinoma was the most common histological type (93.99%) as compared to the adenocarcinoma (6.01%).

Genotyping methodology

Three milliliter peripheral blood sample was collected from each subject in EDTA vial and genomic DNA was extracted using organic method (Adeli and Ogbonna, 1990). Genotyping of *VEGF*−116G/A (rs1570360) polymorphism was done using the Sanger sequencing. Each PCR reaction volume of 25µl contained 100ng of genomic DNA, 1X *Taq* buffer with 1.5mM MgCl2, 0.5 µl of 2.5 mM dNTP mix, 11picomoles forward and reverse primer, 1X Q solution and 0.6U *Taq* polymerase. Purified PCR products were bi directionally sequenced using Big Dye terminator Kit. The methodology used has been detailed in our previous study (Kapahi et al., 2014).

Statistical analysis

The deviation from the Hardy-Weinberg equilibrium among controls was calculated using chi-square goodness-of-fit test ($p>0.05$). The differences in the genotype and allele frequencies between esophageal cancer patients and controls were compared using the chi-square test. The association of *VEGF*-116G/A polymorphism with esophageal cancer risk was assessed using the odds ratio (OR) and their 95% CI. The data analysis was done using online SNPstats software (Sole et

al., 2006) and p value < 0.05 was considered statistically significant.

Prediction of impact of VEGF-116G/A polymorphism on Transcription Factor Binding Sites

The Promo 3 online software (Messeguer et al., 2002) was used to predict the influence of *VEGF*-116G/A polymorphism on transcription factor binding sites.

Meta-analysis

This meta-analysis was performed based on the Preferred Reporting items for Systematic Review and Meta-analysis (PRISMA) guidelines (Moher et al., 2010). The study question was formulated based on the Participant, Intervention, Comparison, Outcomes, and Studies (PICOS) criteria (Eriksen and Frandsen, 2018).

Participants (P)

Patients with cancers in the GIT tract, regardless of age; Healthy control individuals regardless of age, gender and ethnicity.

Intervention (I)

Prevalence of *VEGF*-116G/A polymorphism.

Comparisons (C)

Comparison of *VEGF*-116G/A genotypic frequency among cancer patients and healthy controls.

Outcome (O)

Association of *VEGF*-116G/A polymorphism with GIT cancer risk.

Study design (S)

Case–control studies evaluating the association of *VEGF*-116G/A polymorphism with GIT cancer risk, Full text articles, published in English.

Publication search strategy

All the available published literature as of February 2023 was extracted from various online resources such as ScienceDirect, Google Scholar and PubMed to identify the studies investigating the association between *VEGF*-116G/A polymorphism and GIT cancer risk. The strategy for searching relevant articles included a combination of various keywords which included "GIT", "gastric", "oral", "liver", "hepatocellular", "esophageal", "colorectal", "pancreatic", "colon", "gall bladder", "rectal" "cancer" or "carcinoma", "SNP" or "polymorphism" or "variant", "risk" or "susceptibility", "*VEGFA*" or "vascular endothelial growth factor", "*VEGF*-116G/A" or "*VEGF*-1154G/A" or "rs1570360" We limited our analysis to studies conducted on human subjects and were written in English language. We further refined our search by manually examining the references cited in the retrieved articles to not exclude other relevant articles.

Criteria for exclusion and inclusion of studies

In the present study, the retrieved studies were examined thoroughly and irrelevant articles were excluded.

Inclusion criteria

1. Case-control studies investigating the association of *VEGF*-116G/A polymorphism with cancer susceptibility in GIT cancers.

2. Distribution of genotypes in Hardy-Weinberg equilibrium in control subjects.

3. Adequate information available on genotype and allele frequency for calculation of odds ratio (OR), 95% CI and p-value.

Exclusion criteria

1. Incomplete genotype and allele data

2. Studies with overlapping and duplicate data.

3. Abstracts, letters to editors, case reports, review articles and meta-analyses.

4. Articles not written in a English language, in vitro studies and studies involving animal models.

Selection of studies

Two authors independently reviewed all the primary articles by carefully examining the titles and abstracts of the articles. The irrelevant studies were removed from the analysis and the full text articles were then assessed for eligibility. The studies not meeting the inclusion criteria were excluded from the analysis. The reasons for the exclusion of studies were also recorded according to the PRISMA guidelines. If there was any disagreement in the study analysis between the authors, the final decision was done through discussion among all authors.

Data extraction

The relevant data was carefully extracted from all the selected studies which included the name of the first author, year of publication, number of cases and controls, genotype distribution in both cases and controls, the p-value for Hardy-Weinberg equilibrium, ethnicity and the findings of the individual studies.

Statistical analysis

Online statistical software MetaGenyo was used to perform all the statistical analyses in this meta-analysis (Martorell-Marugan et al., 2017). The association of *VEGF*-116G/A polymorphism with GIT cancer risk was assessed under different genetic models and their OR and 95%CI was calculated. A p-value of <0.05 was considered statistically significant. We determined the heterogeneity (I^2) between the studies using Cochran's Q-test. If the I^2 value is >50% and the p-value <0.05 (presence of heterogeneity), then a random effect model was selected. If heterogeneity was not detected, then the fixed effect model was applied. Forest plots were constructed to display the results in a graphical form. The funnel plots were constructed to visually evaluate the publication bias in the study. Sensitivity analysis was also performed to evaluate the reliability of the results by sequentially deleting each study and then reanalyzing the results.

Results

Case-control study

In the present study, 333 sporadic esophageal cancer

patients (143 males, 190 females) and 346 age and gender-matched healthy controls (149 males, 197 females) were screened for *VEGF*-116G/A polymorphism. The genotype distribution in controls was in Hardy-Weinberg equilibrium (p=0.567). Based on the data obtained, AA genotype (OR=1.88, 95% CI, 1.11-3.19, p=0.02) and A allele (OR= 1.27, 95% CI, 1.01-1.60, p=0.04) of *VEGF*-116G/A polymorphism was associated with an increased risk of esophageal cancer (Table 1). Genetic model analysis showed that *VEGF*-116G/A polymorphism was associated with an increased risk of esophageal cancer under the recessive (OR=1.80, 95% CI, 1.09-2.98, p=0.02) and log additive model (OR=1.27, 95% CI, 1.01-1.59, p=0.04) (Supplementary Table 1). Stratification analysis based on gender revealed no significant association with esophageal cancer risk (Table 1 and Supplementary Table 1).

Prediction of Transcription Factor Binding sites

VEGF promoter DNA sequence containing *VEGF*-116G/A polymorphism was used to predict the impact of *VEGF*-116G/A polymorphism on transcription factors binding sites. It was observed that A allele of *VEGF*-116G/A polymorphism created new binding sites for STAT4, c-Ets-1 and Elk-1 transcription factors (Figure 1).

Meta-analysis

Characteristics of the included studies

The PRISMA flowchart of study selection process has been shown in Figure 2. The characteristics of the selected studies have been briefly summarized in Table 2. A total of ten studies were included in the meta-analysis after applying inclusion and exclusion criteria. Out of the ten

Table 1. Association of *VEGF*-116G/A Polymorphism with Risk to Esophageal Cancer

Genotype/ Allele	Patients n (%)	Controls n (%)	OR (95%CI)	p value
Total				
Genotype				
GG	149 (44.8)	172 (49.7)	Reference	
GA	140 (42.0)	147 (42.5)	1.10 (0.80-1.51)	0.56
AA	44 (13.2)	27 (7.8)	1.88 (1.11-3.19)	0.02*
Allele				
G	438 (65.8)	491 (71.0)	Reference	
A	228 (34.2)	201 (29.0)	1.27 (1.01-1.60)	0.04*
Females				
Genotype				
GG	88 (46.3)	99 (50.3)	Reference	
GA	74 (39.0)	81 (41.1)	1.03 (0.67-1.57)	0.9
AA	28 (14.7)	17 (8.6)	1.85 (0.95-3.61)	0.07
Allele				
G	250 (65.8)	279 (70.8)	Reference	
A	130 (34.2)	115 (29.2)	1.26 (0.93-1.71)	0.13
Males				
Genotype				
GG	61 (42.7)	73 (49.0)	Reference	
GA	66 (46.1)	66 (44.3)	1.20 (0.74-1.94)	0.47
AA	16 (11.2)	10 (6.7)	1.91 (0.81-4.53)	0.14
Allele				
G	188 (65.7)	212 (71.1)	Reference	
A	98 (34.3)	86 (28.9)	1.28 (0.91-1.82)	0.16

*Statistically significant p values

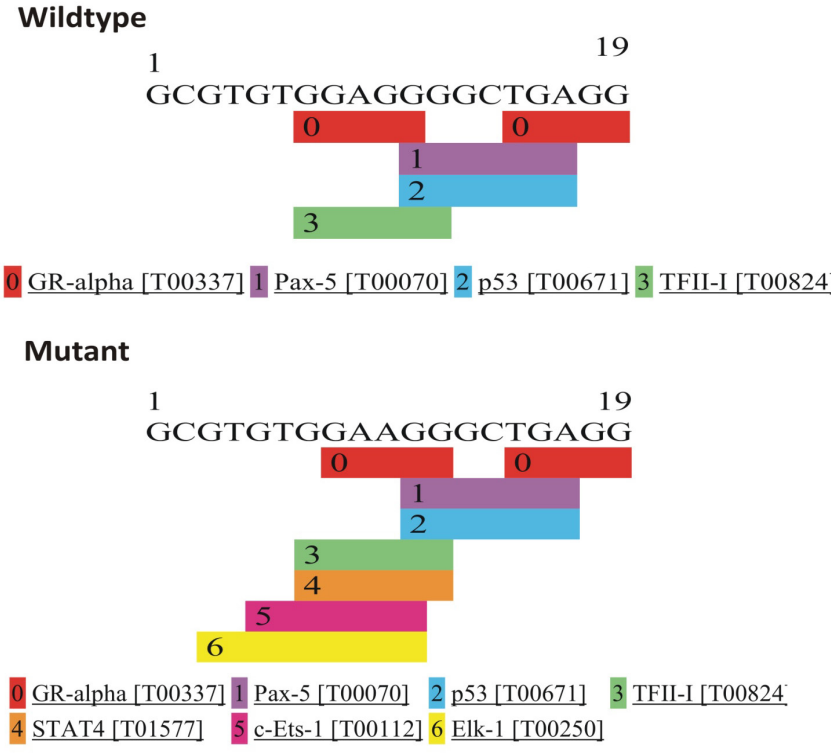

Figure 1. Prediction of Transcription Factor Binding Sites for *VEGF* -116G/A Polymorphism

Table 2. A Brief Summary of Published Studies on *VEGF*-116G/A Polymorphism in GIT Cancers

Study	Cancer	Ethnicity	Patients/ Controls	Patients			Controls			HWE p value	Association	Findings	Study included in the meta analysis
				GG	GA	AA	GG	GA	AA				
Present study	Esophageal	Asian	333/346	149	140	44	172	147	27	0.567	Yes	↑ risk with AA genotype	Yes
Supic et al., 2012	Oral	Caucasian	114/126	46	58	10	61	59	6	0.078	Yes	↓ overall survival with GG genotype	Yes
Kammerer et al., 2010	Oral	Caucasian	80/40	26	41	13	20	14	6	0.2	No		Yes
Tzanakis et al., 2006	Gastric	Caucasian	100/100	45	36	19	42	43	15	0.469	No		Yes
Cacev et al., 2008	Colon	Caucasian	152/156	60	73	19	52	81	23	0.345	No		Yes
Jang et al., 2013	Colorectal	Asian	390/492	279	98	13	349	130	13	0.83	No		Yes
Choi et al., 2011	Colorectal	Asian	278/226	184	66	28	157	60	9	0.29	Yes	↑ risk with A allele in males	Yes
Dassoulas et al., 2009	Colorectal	Caucasian	312/362	126	138	48	152	156	54	0.183	No		Yes
Ungerback et al., 2009	Colorectal	Caucasian	296/332	138	131	27	156	142	34	0.841	No		Yes
Yamamori et al., 2004	Colorectal	Asian	18/-	18	-	-	-	-	-	NC	No		No
Mishra et al., 2013	Gall bladder	Asian	195/300	97	77	21	189	85	26	0.0006	Yes	↑ risk with A allele	No
Baitello et al., 2016	Hepatocellular	Mixed	102/127	61	35	6	73	47	7	0.875	No		Yes
Song et al., 2014	Hepatocellular	Asian	146/-	86	47	13	-	-	-	NC	Yes	↑ risk with AA genotype	No
Wu et al., 2013	Hepatocellular	Asian	HPC#/CHB##								Yes	↓ risk with A allele	No
			101/110	83	17	1	75	31	4	0.003			
			402/1043	304	94	4	685	311	47				
			337/310	219	102	16	171	114	25				
			367/375	266	89	12	247	105	23				
Wu et al., 2009	Hepatocellular	Asian	90/99	66	24	-	72	27	-		No		No

#, Hepatocellular carcinoma patients; ##, Chronic hepatitis B patients; NC, Not calculated

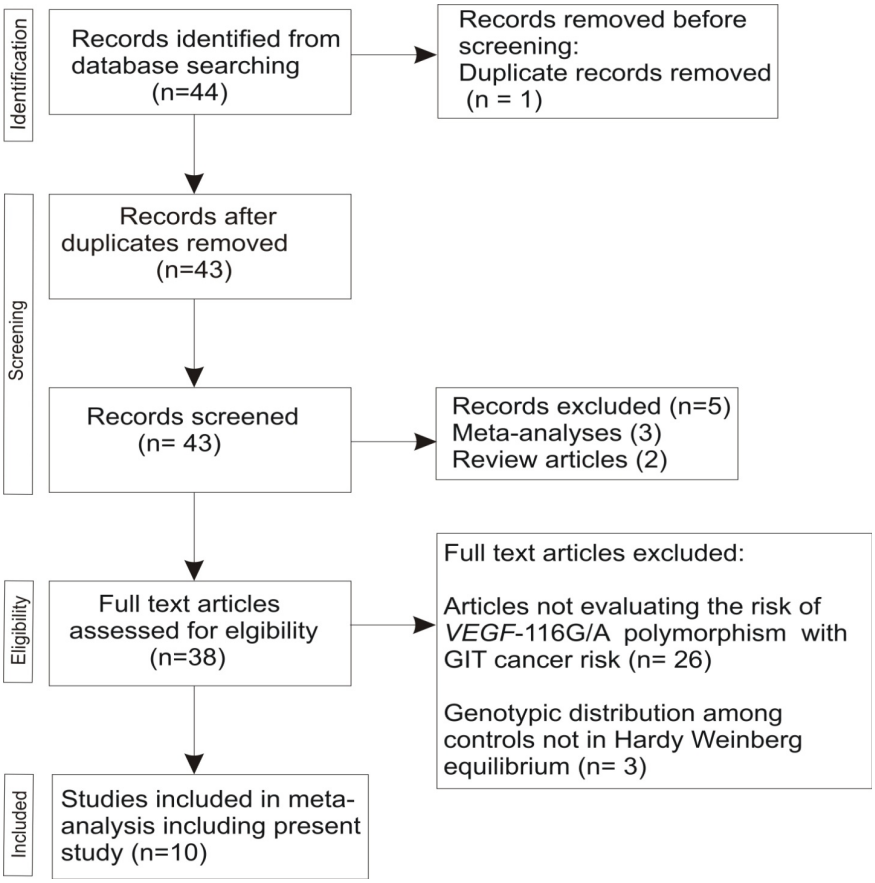

Figure 2. PRISMA Flowchart Demonstrating Process of Selection of Studies

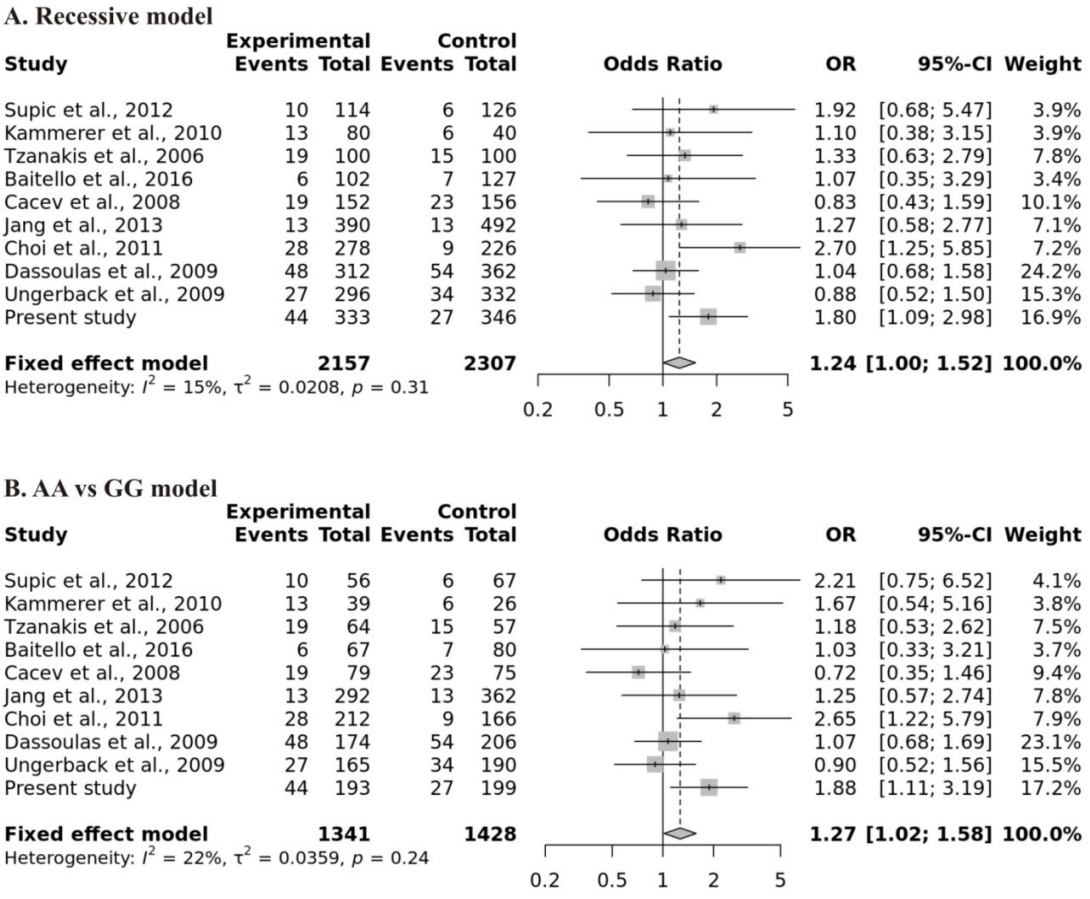

Figure 3. Figure Demonstrating Forest Plot of *VEGF*-116G/A Polymorphism; A, Recessive; B, AA vs GG model

Table 3. Association of *VEGF*-116G/A Polymorphism with GIT Cancer Risk in Different Ethnic Groups

Model	Ethnicity	Number of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95% CI	p value	Model	p value	I^2	Egger's test p value
Allele contrast (A vs G)	Overall	10	1.088	0.990- 1.196	0.079	Fixed	0.355	0.095	0.647
	Asian	3	1.188	1.022- 1.381	0.025*	Fixed	0.271	0.234	0.865
	Caucasian	6	1.035	0.913- 1.174	0.588	Fixed	0.412	0.006	0.293
	Mixed	1	0.947	0.613- 1.463	0.807	Fixed	NA	NA	NA
Recessive model (AA vs AG+GG)	Overall	10	1.237	1.005- 1.522	0.045*	Fixed	0.305	0.15	0.498
	Asian	3	1.826	1.259- 2.648	0.001*	Fixed	0.403	0	0.971
	Caucasian	6	1.034	0.799- 1.337	0.8	Fixed	0.759	0	0.261
	Mixed	1	1.071	0.349-3.294	0.904	Fixed	NA	NA	NA
Dominant model (AA+AG vs GG)	Overall	10	1.066	0.943- 1.204	0.308	Fixed	0.533	0	0.582
	Asian	3	1.103	0.918- 1.326	0.295	Fixed	0.54	0	0.752
	Caucasian	6	1.051	0.885- 1.248	0.569	Fixed	0.281	0.203	0.436
	Mixed	1	0.909	0.535- 1.543	0.723	Fixed	NA	NA	NA
Over dominant (AG vs AA+GG)	Overall	10	0.983	0.869- 1.113	0.789	Fixed	0.765	0	0.611
	Asian	3	0.936	0.774- 1.132	0.493	Fixed	0.879	0	0.296
	Caucasian	6	1.034	0.871- 1.226	0.705	Fixed	0.445	0	0.644
	Mixed	1	0.889	0.516- 1.533	0.673	Fixed	NA	NA	NA
AA vs GG	Overall	10	1.266	1.017- 1.576	0.035*	Fixed	0.244	0.216	0.509
	Asian	3	1.855	1.266- 2.717	0.002*	Fixed	0.41	0	0.956
	Caucasian	6	1.052	0.799- 1.384	0.719	Fixed	0.554	0	0.223
	Mixed	1	1.026	0.327- 3.214	0.965	Fixed	NA	NA	NA
AA vs AG	Overall	10	1.223	0.982- 1.524	0.073	Fixed	0.366	0.082	0.553
	Asian	3	1.805	1.219- 2.671	0.003*	Fixed	0.423	0	0.834
	Caucasian	6	1.016	0.773- 1.335	0.908	Fixed	0.773	0	0.502
	Mixed	1	1.151	0.356- 3.727	0.815	Fixed	NA	NA	NA
AG vs GG	Overall	10	1.016	0.893- 1.156	0.812	Fixed	0.631	0	0.558
	Asian	3	0.997	0.821- 1.211	0.977	Fixed	0.751	0	0.802
	Caucasian	6	1.047	0.873- 1.255	0.621	Fixed	0.294	0.185	0.547
	Mixed	1	0.891	0.512-1.551	0.684	Fixed	NA	NA	NA

*, Statistically significant p value; NA, Not applicable

studies, two studies were on oral cancer, one study each on esophageal, gastric, hepatocellular, colon cancer and four studies on colorectal cancer. Six studies were conducted in Caucasians, three studies on Asians and one study was conducted in mixed population. The genotype distribution in controls in all the included studies was in agreement with the Hardy-Weinberg Equilibrium.

Association of VEGF-116G/A polymorphism with GIT cancer risk

We observed that polymorphism *VEGF*-116G/A was associated with an increased risk of GIT cancer under the recessive (OR= 1.237, 95% CI, 1.005-1.522, p=0.045) and AA vs GG genetic model (OR= 1.266, 95% CI, 1.017-1.576, p=0.035) in the overall population (Table 3). On stratification of studies by ethnicity, we observed an increased risk of GIT cancers in Asians under allele contrast (OR=1.188, 95% CI, 1.022-1.381, p=0.025), recessive (OR=1.826, 95%CI, 1.259-2.648, p= 0.001), AA vs GG (OR=1.855, 95%CI, 1.266-2.717, p=0.002) and AA vs AG model (OR=1.805,95%CI, 1.219-2.671, p=0.003) (Table 3). Subgroup analysis based on cancer type showed an increased risk of esophageal cancer under allele contrast (OR=1.272, 95%CI, 1.011-1.599, p=0.040), recessive (OR=1.799, 95%CI, 1.086-2.980, p=0.023), AA vs GG (OR=1.881, 95%CI, 1.111-3.186, p=0.019) and AA vs AG comparison model (OR=1.711, 95%CI, 1.005-2.913, p=0.048). Similarly, increased risk of oral cancer was observed under the allele contrast model (OR=1.378, 95%CI, 1.002-1.897, p=0.049) and dominant model (OR=1.568, 95%CI, 1.002-2.404, p= 0.039) (Table 4). The results were graphically represented in the form of forest plots (Figure 3).

Table 4. Association of *VEGF*-116G/A Polymorphism with Risk of GIT Cancers

Model	Cancer	Number of studies	Reported OR			Publication bias Egger's test p value
			OR	95% CI	p value	
Allele contrast (A vs G)	Colon	1	0.838	0.605- 1.159	0.285	NA
	Colorectal	4	1.059	0.934- 1.202	0.369	0.178
	Esophageal	1	1.272	1.011- 1.599	0.040*	NA
	Gastric	1	1.022	0.680- 1.534	0.917	NA
	Hepatocellular	1	0.947	0.613- 1.463	0.807	NA
	Oral	2	1.378	1.002- 1.897	0.049*	NA
Recessive model (AA vs AG+GG)	Colon	1	0.826	0.430- 1.588	0.566	NA
	Colorectal	4	1.156	0.871- 1.534	0.316	0.299
	Esophageal	1	1.799	1.086- 2.980	0.023*	NA
	Gastric	1	1.329	0.633- 2.792	0.452	NA
	Hepatocellular	1	1.071	0.349- 3.294	0.904	NA
	Oral	2	1.456	0.694-3.057	0.32	NA
Dominant model (AA+AG vs GG)	Colon	1	0.767	0.481- 1.221	0.263	NA
	Colorectal	4	1.041	0.887- 1.220	0.624	0.106
	Esophageal	1	1.221	0.903- 1.651	0.195	NA
	Gastric	1	0.885	0.506-1.548	0.669	NA
	Hepatocellular	1	0.909	0.535-1.543	0.723	NA
	Oral	2	1.568	1.022- 2.404	0.039*	NA
Over dominant (AG vs AA + GG)	Colon	1	0.856	0.547-1.338	0.494	NA
	Colorectal	4	0.986	0.837- 1.160	0.862	0.317
	Esophageal	1	0.982	0.724- 1.332	0.907	NA
	Gastric	1	0.746	0.422-1.317	0.312	NA
	Hepatocellular	1	0.889	0.516- 1.533	0.673	NA
	Oral	2	1.366	0.892- 2.091	0.151	NA
AA vs GG	Colon	1	0.716	0.351-1.459	0.358	NA
	Colorectal	4	1.188	0.883- 1.598	0.256	0.319
	Esophageal	1	1.881	1.111- 3.186	0.019*	NA
	Gastric	1	1.182	0.533 -2.623	0.68	NA
	Hepatocellular	1	1.026	0.327- 3.214	0.965	NA
	Oral	2	1.931	0.884- 4.218	0.099	NA
AA vs AG	Colon	1	0.917	0.462- 1.818	0.803	NA
	Colorectal	4	1.143	0.847- 1.544	0.382	0.238
	Esophageal	1	1.711	1.005- 2.913	0.048*	NA
	Gastric	1	1.513	0.674- 3.397	0.316	NA
	Hepatocellular	1	1.151	0.356- 3.727	0.815	NA
	Oral	2	1.148	0.525- 2.512	0.729	NA
AG vs GG	Colon	1	0.781	0.480- 1.272	0.321	NA
	Colorectal	4	0.999	0.844- 1.183	0.995	0.782
	Esophageal	1	1.099	0.799- 1.512	0.56	NA
	Gastric	1	0.781	0.424- 1.439	0.428	NA
	Hepatocellular	1	0.891	0.512- 1.551	0.684	NA
	Oral	2	1.521	0.973- 2.377	0.066	NA

*, Statistically significant p values; NA, Not applicable

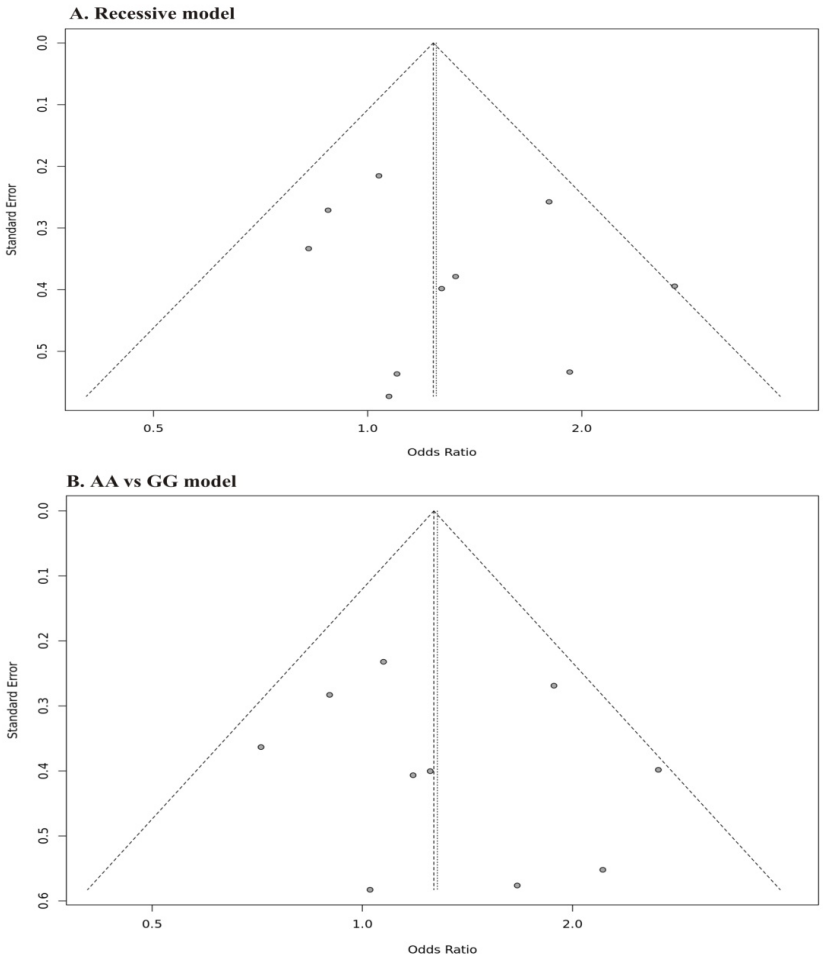

Figure 4. Figure Demonstrating Funnel Plot of *VEGF*-116G/A Polymorphism; A, Recessive; B, AA vs GG model

Heterogeneity test

There was no heterogeneity in any of the genetic models in both overall and subgroup analysis as evidenced by Cochran's Q-test. Hence, we applied the fixed effect model to all genetic models for the statistical analysis.

Sensitivity analysis and publication bias

We did not observe any publication bias in the study as cleared from the symmetry of the funnel plots (Figure 4) and p-value for Egger's test ($p>0.05$ in both overall and subgroup analysis). Sensitivity analysis was performed to confirm the robustness of the study. When we sequentially removed the studies, no significant effect was observed in the meta-analysis results (Supplementary Figure 1).

Discussion

Vascular Endothelial Growth Factor (*VEGF*) is an important pro–angiogenic mitogen which promotes endothelial cell migration and vascular permeability. Dysregulation of the normal activities of the *VEGF* may affect the tumour microenvironment, thus playing an important role in pathological angiogenesis. Genetic polymorphisms in *VEGF* are considered as key regulators required for understanding the molecular mechanisms in tumour angiogenesis. In the present case-control study, the association of *VEGF*-116G/A promoter polymorphism with esophageal cancer risk was evaluated. Our results demonstrated that *VEGF*-116 AA genotype and A allele was significantly associated with an increased risk of esophageal cancer. Till date, no study has reported the association of *VEGF*-116G/A polymorphism with esophageal cancer risk. A few studies have investigated the impact of *VEGF* -116G/A promoter polymorphism in GIT cancer susceptibility in different populations and results were conflicting. *VEGF*-116A allele was associated with an increased risk to colorectal cancer in Korean male patients (Choi et al., 2011) and gall bladder cancer in North Indian patients (Mishra et al., 2013). Association of *VEGF*-116A allele with decreased risk to hepatocellular carcinoma has been reported in Chinese patients (Wu et al., 2013). The *VEGF*-116GG genotype was associated with decreased overall survival in oral cancer in Caucasians (Supic et al., 2012). The major reason for the inconsistency in the results of different case control studies is variability among different populations.

It has been reported that genetic variants in the *VEGF* affect the transcriptional regulation of the gene by altering the binding sites of several transcription factors, thus affecting *VEGF* secretion (Watson et al., 2000).The impact of *VEGF*-116G/A promoter polymorphism on *VEGF* promoter activity and *VEGF* protein levels has been previously investigated in several studies. Baitello et al. reported that the *VEGF*-116A allele was associated

with increased serum VEGF levels in hepatocellular carcinoma (HCC) patients (Baitello et al., 2016). It has been reported that individuals with the *VEGF*-116GG genotype have increased *VEGF* secretion as compared to A allele carriers (Shahbazi et al., 2002). Individuals with the *VEGF*-460C/-116A haplotype had higher promoter activity compared with the -460T/-116G haplotype in HCC cell lines (Wu et al., 2013).

In the present study, we found that A allele of *VEGF*-116G/A polymorphism created binding sites for STAT4, c-Ets-1and Elk-1 transcription factors. STAT4, Ets-1 and Elk-1 transcription factors are known to regulate angiogenesis (Iwasaka et al., 1996; Randi et al., 2009). STAT4 is involved in the transcription of IL8 interleukin, which is involved in the production of several inflammatory mediators (Nguyen et al., 2017) and also involved in tumour metastasis and progression (Zhao et al., 2017). Ets-1 has been reported to be involved in the regulation of several endothelial–specific genes such as *VEGF*, Flk1 and Tie2 and also regulates several extracellular proteases such as MMP9 (Iwasaka et al., 1996). It has been reported that the transcription factor Elk-1 regulates the genes involved in cell migration (Kasza , 2013).

A meta- analysis is considered a more robust approach than individual studies as it increases the statistical significance in genetic association studies (Munafo and Flint, 2004). We combine the results of individual studies on GIT cancers with small sample sizes and performed a meta-analysis to estimate the association of *VEGF*-116G/A with GIT cancer risk. A significant association with the risk of GIT cancer was observed under recessive and AA vs GG genetic model. We also observed an increased risk of GIT cancers only among Asians under allele contrast, recessive, AA vs GG and AA vs AG models. Till date, three meta-analyses has been performed on *VEGF* -116G/A polymorphism in GIT cancers. Metzger et al., (2015) conducted a meta-analysis to investigate the association of the *VEGF*-116 G/A polymorphism with the smoking status in 227 oral squamous cell carcinoma patients and reported no association with risk. A meta-analysis including 100 gastric cancer patients and 100 healthy controls, found no correlation with gastric cancer risk (Zhuang et al., 2017). In another meta-analysis of two case-control studies on colorectal cancer revealed no association of *VEGF*-116G/A polymorphism with cancer risk (Zhou et al., 2011). In contrast, our study on 2,157 patients and 2307 controls reported a significant association with GIT cancer risk.

Our meta-analysis has several advantages. It included a large number of studies on various GIT cancers (n=10) as compared to previously published meta-analyses. However, there are some limitations in our study as well. Gastrointestinal tract cancers arise from a complex interaction between genes and the environment, however, we could not investigate the gene-gene and gene-environment interactions due to lack of data on confounding factors. In conclusion, the findings of present case-control study and meta-analysis demonstrated that *VEGF*-116G/A polymorphism was associated with risk to esophageal as well as other GIT cancers.

Author Contribution Statement

KG and VS designed the study. DM and KG performed the experiments. DM and KG analyzed the data and prepared the manuscript. MSU and MS did clinical diagnosis of patients and also helped in collection of blood samples of patients.

Acknowledgements

We thank the study participants for taking part in the present study.

Funding statement

This study was supported by the MHRD grant sanctioned to Dr Kamlesh Guleria and Dr Vasudha Sambyal under RUSA scheme.

Ethical issue

This study was approved by the institutional ethics committee of Guru Nanak Dev University, Amritsar, Punjab, India.

Availability of data

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

Conflict of interest

All the authors declare that they have no conflict of interest.

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