RESEARCH ARTICLE

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Association of Insertion/Deletion Polymorphisms of *MDM2*, *MCP-1* and *VEGF* with Esophageal Cancer Risk in North-West Indians: A Case - Control Study

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Abstract

Background: The carcinogenesis process in esophageal cancer (EC), a highly heterogeneous and multifaceted disease, is influenced by both tumor angiogenesis and chronic inflammation pathways. Genetic variants in these pathways may affect the progression and development of EC, ultimately contributing to different susceptibilities to cancer among individuals. **Methods:** In this study, a total of 536 subjects were recruited, including 260 EC patients and 276 healthy individuals. The DNA was isolated from the blood samples of the participants using the standard phenol-chloroform method. The three insertion/deletion (ins/del) polymorphisms (*VEGF*-2549 18bp I/D, *MDM2* 40bp I/D, and *MCP-1* 14bp I/D) were screened using the Direct-polymerase chain reaction (PCR) genotyping method. The role of gene-environment interactions on EC risk was assessed using the Multifactor Dimensionality Reduction (MDR) software (version 3.0.2). **Results:** It was observed that the individuals carrying the II genotype and I allele of the *VEGF*-2549 18bp I/D polymorphism, as well as the carriers of the ID and DD genotypes and D allele of the *MCP-1* 14bp I/D polymorphism had a higher risk of developing EC. No association between the *MDM2* 40bp I/D polymorphism and EC risk was reported in this study. Genotype combination analysis revealed an increased EC risk in the carriers of the II-II-II genotype combination of the *VEGF*-2549 18bp I/D, *MDM2* 40bp I/D, and *MCP-1* 14bp I/D polymorphisms compared to those with other genotype combinations. The gene-environment interaction analysis also indicated a strong interaction between lifestyle factors and genetic polymorphisms in influencing EC risk. **Conclusion:** The present study concluded that the *VEGF*-2549 18bp I/D and *MCP-1* 14bp I/D variants were associated with EC risk in the North-west Indians.

Keywords: Esophageal- Cancer- Polymorphism- Angiogenesis- Inflammation- *VEGF- MCP-1- MDM2*

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Introduction

The tumour development is a highly regulated multistep process accompanied by aberrant genetic, epigenetic and environmental changes. These molecular and environmental stresses transform normal healthy cells into rapidly dividing highly heterogeneous malignant cells [1]. Among the various hallmarks of cancer, sustained angiogenesis is recognised as the most important process for metastatic spread and local invasion of the tumour cells. The *VEGF* protein regulates the process of "angiogenic switch" in the tumour cells and also helps in the formation of new blood vasculature required for controlling the oxygen and nutrient demands of growing tumour cells [2,3]. The rapid hyper-proliferation of the tumour cells creates a severe hypoxic microenvironment [4]. Hypoxia induces the stabilization of the HIF1α protein which further promotes the enhanced transcription of the *VEGF* mRNA [5,6]. The upregulated *VEGF* protein interacts with the *VEGFR*2 receptor and promotes the molecular and biological processes required for tumour angiogenesis [7].

MDM2 and *MCP-1* proteins also influence *VEGF*-induced tumour angiogenesis. *MDM2* is an oncogenic protein that regulates hypoxia-dependent *VEGF* expression. It has been reported that along with cellular stresses, environmental stresses such as hypoxia also stimulate *MDM2* overexpression [8]. In hypoxic conditions inside the tumour cells, the *MDM2* protein interacts with the 3' untranslated region (UTR) of the

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VEGF mRNA, stabilizes the *VEGF* mRNA, regulates *VEGF* translation and upregulates its expression [9]. It has been reported that the *MDM2* protein also inhibits the production of anti-angiogenic factors, further promoting tumour angiogenesis [10].

It has been documented that chronic inflammation plays a vital role in tumour development and progression [11]. The *MCP-1* (CCL2) chemokine, a crucial mediator of chronic inflammation, promotes monocyte recruitment and activation [12], secretes monocyte-mediated proinflammatory signals [13] and influence tumour growth, metastasis and angiogenesis [14]. It has been reported that the secretion of high *MCP-1* levels by the tumour cells and tumour-infiltrating monocytes, further increases *VEGF* expression and promotes angiogenesis [15]. In-vitro studies have also documented a strong correlation between high levels of *VEGF* and increased *MCP-1* mRNA expression in the endothelial cells [16]. It has been reported that the hypoxia-inducible factor-1α (HIF-1α) also plays a role in stimulating *VEGF* and *MCP-1*-mediated angiogenesis [15].

Esophageal cancer (EC) is a highly heterogeneous tumour, characterized by its aggressive nature and poor survival. Previous studies have documented the relationship between *VEGF*, *MCP-1* and *MDM2* overexpression with lymph node metastasis, tumour invasion, distant metastasis, and poor prognosis in EC [17-20]. Since hypoxia has been reported as a major regulator of *VEGF*, *MDM2* and *MCP-1* expression, the interplay between these proteins might play an important role in EC development.

Genetic variability is responsible for inter-individual variation in predisposition towards different cancers. Among the various forms of genetic variability, small ins/del (I/D) polymorphisms represent humans' most common form of genetic variation [21]. It has been reported that I/D polymorphisms impact key biological processes in humans and are also implicated in the pathogenesis of several diseases. Functional I/D polymorphisms are known to influence gene function [22]. It has been documented that the I/D polymorphisms located in the promoter region affect gene expression variability among humans. The two important I/D polymorphisms located in the promoter region are *VEGF*-2549 18bpI/D (rs35569394) and *MDM2* 40bpI/D (rs3730485) polymorphism. The *VEGF*-2549I/D polymorphism is an 18bp I/D polymorphism located at -2549 upstream of the *VEGF* promoter. It has been reported that the *VEGF*-2549 18bp I/D polymorphism influences the *VEGF* promoter activity as well as *VEGF* protein production [23,24]. The *MDM2* 40bpI/D polymorphism is a 40bp I/D polymorphism, situated at the -1518 position upstream of the constitutive P1 promoter [25, 26] of the gene. Association of *MDM2* 40bp I/D polymorphism with altered transcriptional activity been previously reported in in-vitro analysis [27]. The role of *VEGF*-254918bp I/D polymorphism in influencing cancer susceptibility has been studied in various gastrointestinal tract (GIT) cancers including esophageal [28], gastric [29], hepatocellular [30], gall bladder [31] and colorectal cancer [32]. The *MDM2* 40bp I/D polymorphism has been studied in GIT cancers including esophageal [33-35], gastric [33, 36], hepatocellular [37], colon [38] and colorectal [36] cancer.

The *MCP-1* 14bp I/D polymorphism is a 14bp I/D polymorphism (rs3917887) present in intron 1 (int1del554-567) of the *MCP-1* [39]. Polymorphisms located in the intronic regions of the gene might also affect gene expression levels by affecting the splicing process [40]. Till now, the relationship between *MCP-1* 14bp I/D polymorphism and cancer susceptibility has been reported from India in the prostate [41] and bladder cancer patients [42].

The process of angiogenesis and inflammation, both play a crucial role in EC development. The variants in the genes involved in these pathways might deregulate cellular homeostasis, and promote tumour development and progression. Therefore, the present study aims to study the role of three I/D polymorphisms (*VEGF*-2549 18bpI/D, *MDM2* 40bpI/D and *MCP-1* 14bp I/D) in influencing EC risk in patients from Punjab, North-West India. The present study is the first reported study to evaluate the combined role of three I/D polymorphisms of *MCP-1*, *VEGF* and *MDM2* in EC in North-West Indians.

Materials and Methods

Study Type, Sample Collection and DNA Extraction

From January 2021 to December 2023, two hundred and sixty clinically and histologically confirmed EC patients who were referred at Sri Guru Ram Das Institute of Medical Sciences, Amritsar, Punjab, India were recruited in this study. The included patients were sporadic cancer patients who had not received any medical treatment, were not on blood transfusion and were not suffering from any other chronic disease. At the same time, 276 healthy cancer-free controls were recruited in this study. The inclusion criteria for the controls were age, gender, habitat and ethnicity-matched unrelated healthy individuals, free from any chronic disease and not having any family history of any cancer. All the study participants belonged to the Amritsar district of Punjab state or its surrounding areas. The study was constructed following Helsinki Declaration guidelines and the institutional ethics committee of Guru Nanak Dev University, Amritsar approved the study protocol. Before collecting the blood samples, written consent was obtained from all the study participants. The demographic and the clinical details of each study participant were recorded on the pre-designed proforma. In a sterilized EDTA-coated vial, 5ml venous blood sample was collected from each study participant by the trained personnel using sterilized disposable syringes. The collected blood samples were transferred to the laboratory and were stored at -20°C till further use. Isolation of the genomic DNA from the peripheral blood samples was done using the standard phenol-chloroform method [43]. The quality and quality of the DNA samples were checked on 1% agarose gel.

Genotyping of Polymorphisms

Genotyping of *VEGF*-254918bpI/D (rs35569394), *MDM2* 40bpI/D (rs3730485) and *MCP-1* 14bpI/D (rs3917887) polymorphisms was done using the Direct-PCR technique. The targeted region of the studied

polymorphisms was amplified using previously published primer sequences [44-46]. The PCR amplification was carried out using 1 µl of 10X Taq buffer with 15mM MgCl2, 0.4 µl of dNTP's mix, 0.4µl of forward and reverse primer (10pmoles/microlitre), 0.9 U of Taq polymerase and 50ng DNA in a 10 µl reaction volume.

The amplification was carried out in T100 thermal cycler (Biorad) at initial denaturation 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing for *VEGF*-254918bpI/D, *MDM2* 40bpI/D, and *MCP-1*14bpI/D polymorphisms were 55°C, 58°C and 56°C respectively and extension at 72°C for 45 seconds and final extension at 72°C for 10 minutes. The PCR products were separated on 2.6% agarose gel and visualized under the UV transilluminator. Genotyping was performed based on the amplicon sizes: *VEGF*-2549 18bp I/D (I allele-229 bp; D allele-211bp), *MDM2* 40bpI/D (I allele-287bp; D allele-247bp) and *MCP-1*14bpI/D (I allele-195bp; D allele-181 bp). The 10% of the results were validated by Sanger sequencing and 100% concordance was observed (Figure 1).

Statistical analysis

The deviation from the Hardy-Weinberg equilibrium (HWE) in control subjects was calculated using the Chi-square test. A p-value of >0.05 was considered statistically significant. The association between the polymorphisms and EC risk was studied by calculating the Odds ratio (OR) and its 95% Confidence Intervals (CI) using the MedCalc software. The genetic model analysis was performed using the online SNPstats software [47]. The results were considered significant if the p-value was less than 0.05.

The Multifactor dimensionality reduction (MDR) (version 3.0.2) software was used to study the overall impact of various interactions between the studied polymorphisms and confounding factors like gender, smoking status, diet and alcohol consumption on EC risk. The model with the maximum testing accuracy, training accuracy and cross-validation consistency was considered the best-studied model compared with other tested SNP models. The synergistic role of the polymorphisms and various confounding factors was examined by the construction of a circle graph based on the interaction entropy model.

Results

Demographic and Clinical Characteristics of the Study Participants

The present case-control study enrolled 260 sporadic EC patients (119 males and 141 females) and 276 healthy age and gender-matched subjects (134 males and 142 females). The mean age of the patients and controls were 56.58±13.24 and 54.03±15.59 years respectively. Of all the patients, 62.3% were older than 50 years. The majority of the EC patients (77.7%) lived in the rural areas of Punjab. There was a higher frequency of vegetarians as compared to non-vegetarians in both EC patients and controls. The frequency of smokers was higher in males EC patients than the male controls. Among patients, majority of the males were farmers, involved in agriculture-related activities or were labourers, whereas females were mostly housewives. Among 141 EC females, 79.4% were postmenopausal whereas 20.6% were pre-menopausal. In this study, squamous cell carcinoma was the most common histological subtype (88.1%), with the majority of the patients having stage II tumours (38.1%). The demographic and clinical characteristics of the study participants have been mentioned in Supplementary Table 1.

Association of VEGF-254918bpI/D Polymorphism with EC Risk

The genotype distribution of the *VEGF*-254918bp I/D polymorphism was in agreement with HWE in the control group (p=0.09). In the total subjects, it was observed that the individuals carrying the II genotype (OR=1.73, 95%CI=1.03-2.91; p=0.04) and I allele (OR=1.28, 95%CI=1.00-1.62; p=0.04) of *VEGF*-2549 18bpI/D polymorphism were more susceptible to EC development (Table 1). The genetic model analysis also revealed a higher predisposition toward EC development under the log-additive genetic model (OR=1.31, 95%CI=1.02-1.69; p=0.04) of *VEGF*-2549 18bp I/D polymorphism (Supplementary Table 2).

The data was stratified based on the gender and female-specific association was observed. The II genotype (OR=2.23, 95%CI=1.07-4.64; p=0.03) was associated with a higher risk of EC in females subjects only (Table 1). The *VEGF*-2549 18bp I/D polymorphism was associated with higher EC risk under the recessive (p=0.04) and log-additive (p=0.04) genetic model in female group (Supplementary Table 2). No genotype was associated with EC risk in male group (p>0.05) (Table 1 and Supplementary Table 2). Age-wise stratification analysis also did not reveal any association between *VEGF*-2549 18bp I/D polymorphism and EC risk (Supplementary Table 3).

Association of MDM2 40bpI/D Polymorphism with EC Risk

The genotype distribution of the *MDM2* 40bpI/D polymorphism was in agreement with HWE in the control group (p=0.34). There was no association of *MDM2* 40bp I/D polymorphism with EC risk in the total, males and females subjects (Table 1 and Supplementary Table 2). The data was further stratified based on the age of the EC patients and no association was observed (p>0.05) (Supplementary Table 3).

Association of MCP-1 14bpI/D Polymorphism with EC Risk

The genotype distribution of *MCP-1* 14bpI/D polymorphism was in agreement with the HWE in the control group (p=0.51). In the total subjects, the carriers of the ID (OR=1.51, 95%CI=1.06-2.15; p=0.02), DD genotype (OR=2.40, 95%CI=1.19-4.81; p=0.01) and D allele (OR=1.50, 95%CI=1.15-1.97; p=0.003) were more susceptible towards EC development (Table 1). Genetic model analysis revealed a significantly higher EC risk under dominant (p=0.006), recessive (p=0.04) and log-

Table 1. Association of *VEGF*-254918bpI/D, *MDM2* 40bp I/D and *MCP-1* 14bpI/D Polymorphism with EC Risk

	Total =536				Females=283				Males=253			
Genotype/Allele	Patients n (%)	Controls n (%)	OR(95%CI)	p-value	Patients n (%)	Controls n (%)	OR(95%CI)	p-value	Patients n (%)	Controls n (%)	OR(95%CI)	p-value
VEGF-254918bp I/D (rs35569394)												
DD	65 (25.0)	87 (31.5)	Reference		31 (22.0)	41 (28.9)	Reference		34 (28.6)	46 (34.3)	Reference	
ID	142 (54.6)	148 (53.6)	1.28(0.86-1.91)	0.21	78 (55.3)	82 (57.8)	1.25 (0.72-2.20)	0.42	64 (53.8)	66 (49.2)	1.32 (0.75-2.30)	0.34
II	53 (20.4)	41 (14.9)	1.73(1.03-2.91)	**0.04**	32 (22.7)	19 (13.4)	2.23 (1.07-4.64)	**0.03**	21 (17.6)	22 (16.4)	1.29 (0.61-2.71)	0.5
D	272 (52.3)	322 (58.3)	Reference		140 (49.6)	164 (57.7)	Reference		132 (55.5)	158 (59.0)	Reference	
I	248 (47.7)	230 (41.7)	1.28(1.00-1.62)	**0.04**	142 (50.4)	120 (42.3)	1.39 (0.99-1.94)	0.05	106 (44.5)	110 (41.0)	1.15 (0.81-1.64)	0.43
MDM2 40bpI/D (rs3730485)												
II	155 (59.6)	160 (58.0)	Reference		87 (61.7)	82 (57.8)	Reference		68 (57.1)	78 (58.2)	Reference	
ID	92 (35.4)	104 (37.7)	0.91(0.64-1.30)	0.62	49 (34.8)	51 (35.9)	0.91 (0.55-1.49)	0.69	43 (36.1)	53 (39.5)	0.93 (0.55-1.56)	0.78
DD	13 (5.0)	12 (4.3)	1.12(0.49-2.53)	0.79	5 (3.5)	9 (6.3)	0.52 (0.17-1.63)	0.26	8 (6.7)	3 (2.2)	-	
I	402 (77.3)	424 (76.8)	Reference		223 (79.1)	215 (75.7)	Reference		179 (75.2)	209 (78.0)	Reference	
D	118 (22.7)	128 (23.2)	0.97(0.73-1.29)	0.85	59 (20.9)	69 (24.3)	0.82 (0.55-1.22)	0.34	59 (24.8)	59 (22.0)	1.17 (0.77-1.76)	0.46
MCP1 14bp I/D(rs3917887)												
II	117 (45.0)	157 (56.9)	Reference		65 (46.1)	85 (59.9)	Reference		52 (43.7)	72 (53.7)	Reference	
ID	118 (45.4)	105 (38.0)	1.51(1.06-2.15)	**0.02**	66 (46.8)	48 (33.8)	1.80 (1.10-2.94)	**0.02**	52 (43.7)	57 (42.5)	1.26 (0.75-2.12)	0.37
DD	25 (9.6)	14 (5.1)	2.40(1.19-4.81)	**0.01**	10 (7.1)	9 (6.3)	1.45 (0.56-3.78)	0.44	15 (12.6)	5 (30.6)	4.15 (1.42-12.14)	**0.009**
I	352 (67.7)	419 (75.9)	Reference		196 (69.5)	218 (76.8)	Reference		156 (65.5)	201 (75.0)	Reference	
D	168 (32.3)	133 (24.1)	1.50(1.15-1.97)	**0.003**	86 (30.5)	66 (23.2)	1.45 (0.99-2.11)	0.05	82 (34.5)	67 (25.0)	1.58 (1.07-2.32)	**0.02**

OR, Odds Ratio; CI,Confidence Interval; p, probability value; significant p values are displayed in bold

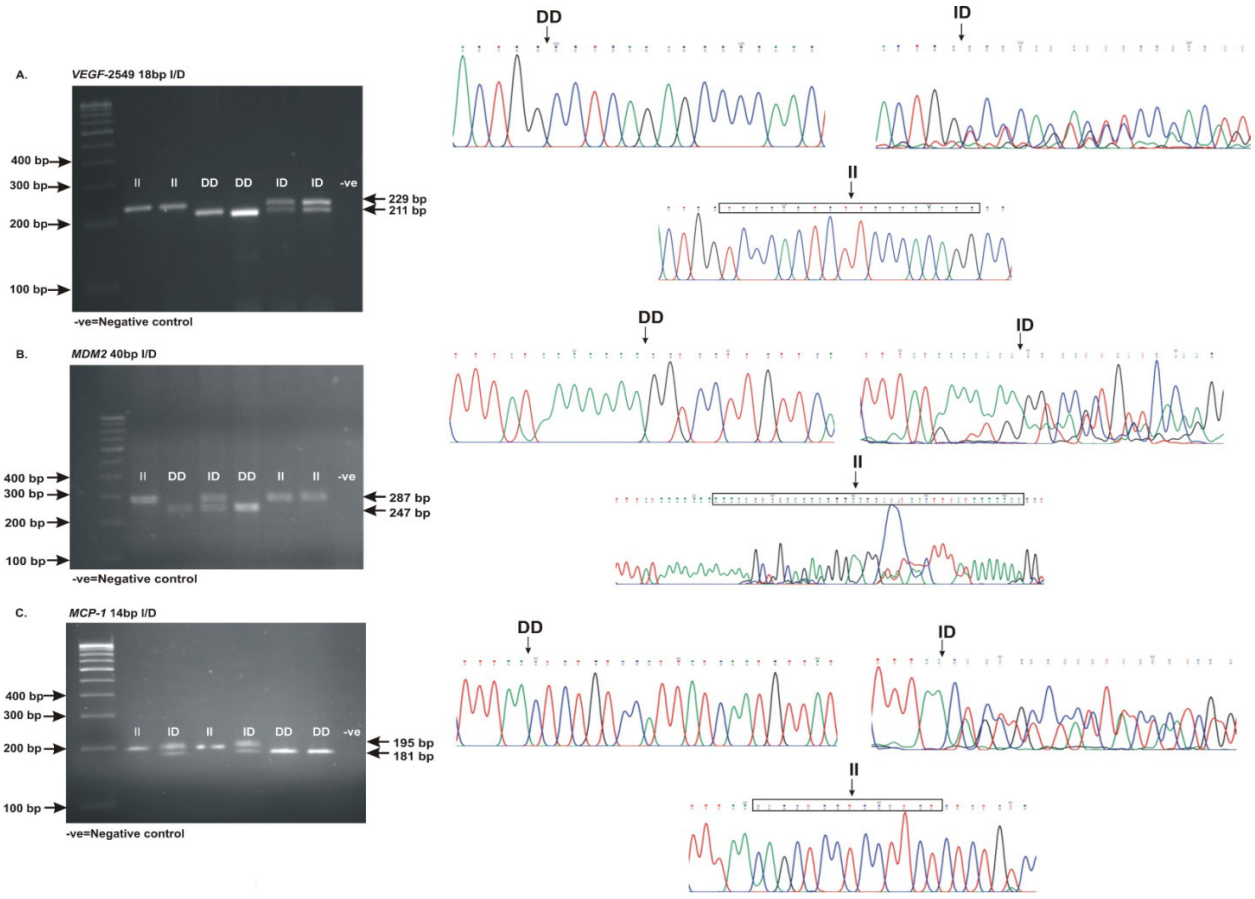

Figure 1. The Gel Photograph and Electropherograms Showing Different Genotypes of *VEGF* -2549 18bp I/D (A), *MDM2* 40bp I/D (B) and *MCP-1* 14bp I/D (C) Polymorphisms

additive model (p=0.002) (Supplementary Table 2).

In female group, increased EC risk was observed with the ID genotype (OR=1.80, 95%CI=1.10-2.94; p=0.02) (Table 1). The *MCP-1* 14bp I/D polymorphism was associated with higher EC risk in females under the dominant (p=0.02), overdominant (p=0.03) and log-additive genetic model (p=0.04) (Supplementary Table 2).

In males, carriers of the DD genotype (OR=4.15, 95%CI=1.42-12.14; p=0.009) and D allele (OR=1.58, 95%CI=1.07-2.32; p=0.02) were more susceptible towards EC development (Table 1). Genetic model analysis revealed

Table 2. Genotype Combinations of *VEGF*-2549 18bpI/D, *MDM2* 40bp I/D and *MCP-1* 14bp I/D Polymorphisms and EC Risk

Genotype Combination	Patients n	Controls n	OR (95%CI)	p-value
VEGF-2549 18bpI/D-*MDM2* 40bpI/D-*MCP1* 14bpI/D				
DD-II-II	15	24	Reference	
DD-ID-ID	10	12	1.33 (0.46-3.84)	0.59
DD-ID-II	11	25	0.70 (0.27-1.84)	0.47
DD-II-ID	20	14	2.29 (0.89-5.85)	0.08
ID-ID-ID	22	23	1.53 (0.64-3.65)	0.34
ID-ID-II	25	22	1.82 (0.77-4.31)	0.17
ID-II-DD	9	5	2.88 (0.81-10.25)	0.1
ID-II-ID	38	32	1.90 (0.86-4.22)	0.12
ID-II-II	33	62	0.85 (0.39-1.84)	0.68
II-ID-ID	6	9	1.07 (0.32-3.61)	0.92
II-ID-II	12	10	1.92 (0.67-5.53)	0.23
II-II-ID	14	9	2.49(0.86-7.16)	0.09
II-II-II	19	10	3.04 (1.12-8.27)	**0.03**

OR, Odds Ratio; CI, Confidence Interval; significant p-values are displayed in bold

Table 3. Gene-Environment Interaction Model

Model	Training balanced Accuracy	Testing balance Accuracy	Cross-validation consistency	OR (95%CI)	p-value
MCPI/D	0.5599	0.5468	9/10	1.61 (1.14-2.27)	**0.006**
MCPI/D, Smoking	0.5857	0.5396	5/10	1.97 (1.40-2.78)	**0.0001**
Gender, Diet, Smoking	0.6204	0.574	7/10	2.99 (2.05-4.36)	**<0.0001**
VEGF-2549I/D,MDM2I/D,MCPI/D, Diet	0.6539	0.5451	8/10	3.37 (2.36-4.80)	**<0.0001**
VEGF-2549I/D,MDM2I/D,MCPI/D, Gender, Diet	0.705	0.5759	10/10	5.41 (3.74-7.83)	**<0.0001**
VEGF-2549I/D,MDM2I/D,MCPI/D, Gender, Diet, Smoking	0.74	0.606	10/10	7.69 (5.24-11.30)	**<0.0001**
VEGF-2549I/D,MDM2I/D,MCPI/D, Gender, Diet, Alcohol, Smoking	0.7498	0.6052	10/10	8.5 (5.76-12.54)	**<0.0001**

OR, Odds Ratio; CI, Confidence Interval; significant p-values are displayed in bold

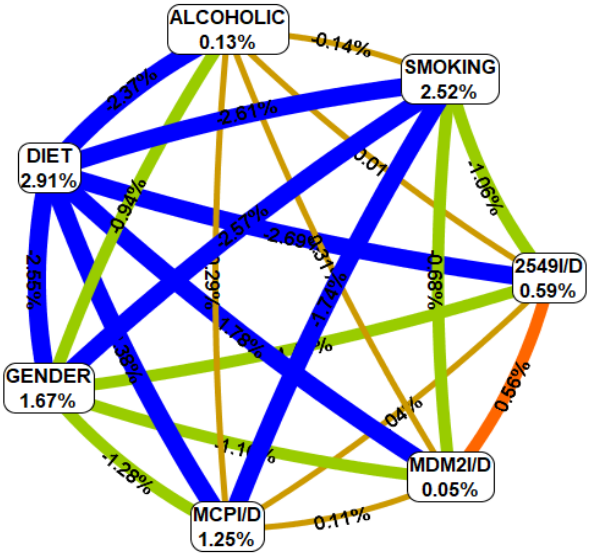

Figure 2. The Circle Graph Depicting the Interaction Entropy Model of EC Patients and Controls, The Degree of Interaction Represented by Different Colours: orange (moderate interaction), green (weak interaction), blue (redundancy), gold (midway interaction between synergy and redundancy

an increased EC risk under the recessive (p=0.008) and log-additive genetic model (p=0.02) (Supplementary Table 2). The genotype data was stratified based on the age at diagnosis of EC patients and no association was observed (p>0.05) (Supplementary Table 3).

Interaction Analysis

To study the combined impact of *VEGF*-2549 18bp I/D, *MDM2* 40bp I/D and *MCP-1* 14bp I/D polymorphisms on EC risk, different genotype combinations were prepared. It was observed that the carriers of II-II-II genotype combination of the *VEGF*-2549 18bpI/D, *MDM2* 40bp I/D and *MCP-1* 14bp I/D polymorphisms had a three-fold higher risk of EC (OR=3.04, 95%CI=1.12-8.27; p=0.03) as compared to the carriers of the other genotype combinations (Table 2).

Gene-Environment Interactions

The interaction between the *VEGF*-2549 18bpI/D, *MDM2* 40bp I/D and *MCP-1* 14bp I/D polymorphisms and confounding factors including gender, diet, alcohol and smoking in determining EC risk was studied. It was observed that the training balance accuracy was highest in the seven-locus model (*VEGF*-2549 18bpI/D, *MDM2* 40bpI/D, MCP 14bp I/D, gender, diet, alcohol, smoking). The testing balance accuracy was highest in the six-locus model (*VEGF*-2549 18bpI/D, *MDM2* 40bp I/D, *MCP-1* 14bp I/D, gender, diet and smoking), suggesting that this model was the best-predicted model for studying the interaction between polymorphisms and lifestyle factors on EC risk and development. All the studied models were statistically significant (p<0.05) (Table 3).

The circle graph was constructed and it showed that the *MCP-1* 14bp I/D polymorphism had the highest entropy (1.25%), contributing the maximum to the development of EC. Also, the interaction entropy model suggested a moderate interaction between the *VEGF*-2549 18bpI/D and *MDM2* 40bp I/D polymorphism in determining the EC risk (0.56%) (Figure 2).

Discussion

The tumour-induced angiogenesis and chronic inflammation play a key role in EC development. In EC, angiogenesis is a prerequisite for the invasion and metastatic growth of the tumour [48]. Esophageal injury caused by environmental exposures induces chronic inflammation in esophageal tissues [49]. This esophageal damage further induces DNA damage and creates genomic instability in the esophageal tissues [50]. Chronic inflammation has been known to promote hypoxic conditions inside the tumour and further influences tumour growth, development and angiogenesis [51]. Genetic variants in the multiple low penetrant susceptibility genes and environmental risk factors influence the development of cancers. Variants in the genes involved in the angiogenesis and chronic inflammation pathways might impact the inter-individual variability in cancer development and progression.

In the present study, it was observed that the

II genotype and I allele of *VEGF*-2549 18bp I/D polymorphism and ID, DD genotype and D allele of *MCP-1* 14bpI/D polymorphism were associated with a higher risk of EC, whereas *MDM2* 40bp I/D polymorphism was not associated with risk of EC in the studied population. In-vitro functional analysis has reported a correlation between the II genotype of *VEGF*-2549I/D polymorphism with enhanced *VEGF* production [24, 52]. The D allele of the *MDM2* 40bp I/D polymorphism has also been associated with reduced *MDM2* expression in cell lines [27]. The role of *MCP-1* 14bp I/D intronic polymorphism on the transcriptional activity of the gene has been reported in earlier study [53]. In-vitro study has reported that *MCP-1* -362C/int1del554-567 Del combination was associated with low *MCP-1* expression [54]. The genetic variability in the *VEGF*, *MDM2* and *MCP-1* genes might affect the regular cellular functions and foster inter-individual susceptibility to EC development.

Several case-control studies in different ethnic populations have evaluated the role of *VEGF*-2549I/D polymorphism with EC susceptibility with inconsistent results. The DD genotype and D allele of *VEGF*-2549 18bp I/D polymorphism has been associated with higher EC risk in the total subjects as well as in female group [28]. Apart from EC, the association of *VEGF*-2549 18bp I/D polymorphism with the risk of other GIT cancers have been studied in different populations. The ID, II genotypes and I allele were associated with higher gastric cancer risk in the South-Indians [29]. The *VEGF*-2549I/D polymorphism was not associated with gall bladder cancer risk in North Indians [31], hepatocellular cancer risk in Han Chinese [30] and colorectal cancer risk in the Swedish population [32]

Similar to our results, no association of *MDM2* 40bp I/D polymorphism has been reported with EC risk in the Chinese population [33-35]. The ID and DD genotypes were associated with lower gastric cancer risk [33] and higher hepatocellular cancer risk [37] in the Chinese population. The II genotype was only associated with lower gastric cancer risk [36] but not with colorectal cancer risk in Brazilians [36]. The association of the D allele with an increased risk of colon cancer has been reported in the Norwegian population [38].

In our study, ID, DD genotype and D allele of *MCP-1* 14bp I/D polymorphism were associated with elevated EC risk in North- Indians. The ID genotype and combined ID+DD genotypes were associated with a higher risk of prostate [41] and bladder cancer [42] in the North Indians. Interaction analysis revealed that the II-II-II genotype combinations of the *VEGF*-2549 18bp I/D, *MDM2* 40bp I/D and *MCP-1* 14bp I/D were associated with higher EC risk. Although *MDM2* alone was not associated with EC risk in the overall analysis, the interaction analysis suggested that *MDM2* I/D polymorphisms might influence EC development in interaction with other polymorphisms. Gene-environment interaction analysis revealed the importance of lifestyle factors in the development of EC. It was revealed in our study that lifestyle factors exhibited strong interactions with other genetic factors in determining EC risk. This is evidenced in a study by Zhai et al. which reported the role of *VEGF*-460T/C polymorphism in influencing EC risk based on the smoking status of the study participants [55].

The variants in these genes affect the clinical response and prognosis of the cancer patients. It has been reported that the DD genotype of *VEGF*-2549 18bp I/D polymorphism has been linked with complete or partial response to chemotherapy in the Chinese EC patients [28] and associated with better treatment response and longer progression free survival in colorectal Caucasian patients [56]. The association of *MDM2* 40bp I/D polymorphism with clinical features and therapy response was studied in Sudanese chronic lymphocytic leukemia patients, but no association was reported [57]. Every cancer patient responds differently to cancer treatment, therefore the variants in these genes could help in early selection of the patients more likely to benefit from a particular treatment plan.

In conclusions, the main limitation of the present case-control study was its limited sample size. Stratification analysis could not be performed because of the limited data on the confounding factors. The present study did not evaluate the functional role of the polymorphisms in influencing EC risk. Understanding the role of other functional polymorphisms in the studied genes will be beneficial in studying the pathology behind EC development and in designing individualized treatment therapies for the EC patients. As India is home to several ethnic groups and different socioeconomic traditions, the impact of the studied polymorphisms on EC risk must be validated in other ethnic groups as well to provide nationwide-based findings.

Author Contribution Statement

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

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Ethical Clearance

This work was approved by Institutional Ethics Committee of Guru Nanak Dev University, Amritsar and some part of this work is a part of Ph.D thesis.

Availability of data and materials

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

Conflict of interest

The authors declare that they have no conflict of interest

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70. https://doi.org/10.1016/s0092-

8674(00)81683-9.
2. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285(21):1182-6. https://doi.org/10.1056/NEJM197111182852108.
3. Nishida N, Yano H, Nishida T, Kamura T, Kojiro M. Angiogenesis in cancer. Vasc Health Risk Manag. 2006;2(3):213-9. https://doi.org/10.2147/vhrm.2006.2.3.213.
4. Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia (Auckl). 2015;3:83-92. https://doi.org/10.2147/HP.S93413.
5. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature. 1992;359(6398):843-5. https://doi.org/10.1038/359843a0.
6. Ziello JE, Jovin IS, Huang Y. Hypoxia-Inducible Factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. Yale J Biol Med. 2007;80(2):51-60.
7. Shibuya M. Vascular Endothelial Growth Factor (*VEGF*) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. Genes Cancer. 2011;2(12):1097-105. https://doi.org/10.1177/1947601911423031.
8. Zhang L, Hill RP. Hypoxia enhances metastatic efficiency by up-regulating Mdm2 in KHT cells and increasing resistance to apoptosis. Cancer Res. 2004;64(12):4180-9. https://doi.org/10.1158/0008-5472.CAN-03-3038.
9. Zhou S, Gu L, He J, Zhang H, Zhou M. *MDM2* regulates vascular endothelial growth factor mRNA stabilization in hypoxia. Mol Cell Biol. 2011;31(24):4928-37. https://doi.org/10.1128/MCB.06085-11.
10. Zafar A, Khan MJ, Naeem A. MDM2- an indispensable player in tumorigenesis. Mol Biol Rep. 2023;50(8):6871-6883. https://doi.org/10.1007/s11033-023-08512-3.
11. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539-45. https://doi.org/10.1016/S0140-6736(00)04046-0.
12. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (*MCP-1*): an overview. J Interferon Cytokine Res. 2009;29(6):313-26. https://doi.org/10.1089/jir.2008.0027.
13. Mukaida N, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. Cytokine Growth Factor Rev. 1998;9(1):9-23. https://doi.org/10.1016/s1359-6101(97)00022-1.
14. Craig MJ, Loberg RD. CCL2 (Monocyte Chemoattractant Protein-1) in cancer bone metastases. Cancer Metastasis Rev. 2006;25(4):611-9. https://doi.org/10.1007/s10555-006-9027-x.
15. Hong KH, Ryu J, Han KH. Monocyte chemoattractant protein-1-induced angiogenesis is mediated by vascular endothelial growth factor-A. Blood. 2005;105(4):1405-7. https://doi.org/10.1182/blood-2004-08-3178.
16. Marumo T, Schini-Kerth VB, Busse R. Vascular endothelial growth factor activates nuclear factor-kappaB and induces monocyte chemoattractant protein-1 in bovine retinal endothelial cells. Diabetes. 1999;48(5):1131-7. https://doi.org/10.2337/diabetes.48.5.1131.
17. Mathew R, Arora S, Khanna R, Mathur M, Shukla NK, Ralhan R. Alterations in p53 and pRb pathways and their prognostic significance in oesophageal cancer. Eur J Cancer. 2002;38(6):832-41. https://doi.org/10.1016/s0959-8049(02)00007-2.
18. Kleespies A, Guba M, Jauch KW, Bruns CJ. Vascular endothelial growth factor in esophageal cancer. J Surg Oncol. 2004;87(2):95-104. https://doi.org/10.1002/jso.20070.
19. Koide N, Nishio A, Sato T, Sugiyama A, Miyagawa S. Significance of macrophage chemoattractant protein-1 expression and macrophage infiltration in squamous cell carcinoma of the esophagus. Am J Gastroenterol. 2004;99(9):1667-74. https://doi.org/10.1111/j.1572-0241.2004.30733.x.
20. Takahashi K, Miyashita M, Makino H, Akagi I, Orita H, Hagiwara N, et al. Expression of Akt and Mdm2 in human esophageal squamous cell carcinoma. Exp Mol Pathol. 2009;87(1):42-7. https://doi.org/10.1016/j.yexmp.2008.11.013.
21. Mills RE, Luttig CT, Larkins CE, Beauchamp A, Tsui C, Pittard WS, et al. An initial map of insertion and deletion (INDEL) variation in the human genome. Genome Res. 2006;16(9):1182-90. https://doi.org/10.1101/gr.4565806.
22. Mullaney JM, Mills RE, Pittard WS, Devine SE. Small insertions and deletions (INDELs) in human genomes. Hum Mol Genet. 2010;19(R2):R131-6. https://doi.org/10.1093/hmg/ddq400.
23. Yang B, Cross DF, Ollerenshaw M, Millward BA, Demaine AG. Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patients with type 1 diabetes mellitus. J Diabetes Complications. 2003;17(1):1-6. https://doi.org/10.1016/s1056-8727(02)00181-2.
24. Salvarani C, Boiardi L, Casali B, Olivieri I, Cantini F, Salvi F, et al. Vascular endothelial growth factor gene polymorphisms in Behçet's disease. J Rheumatol. 2004;31(9):1785-9.
25. Hu Z, Ma H, Lu D, Qian J, Zhou J, Chen Y, et al. Genetic variants in the *MDM2* promoter and lung cancer risk in a Chinese population. Int J Cancer. 2006;118(5):1275-8. https://doi.org/10.1002/ijc.21463.
26. Ma H, Hu Z, Zhai X, Wang S, Wang X, Qin J, et al. Polymorphisms in the *MDM2* promoter and risk of breast cancer: a case-control analysis in a Chinese population. Cancer Lett. 2006;240(2):261-7. https://doi.org/10.1016/j.canlet.2005.09.019.
27. Lalonde ME, Ouimet M, Larivière M, Kritikou EA, Sinnett D. Identification of functional DNA variants in the constitutive promoter region of MDM2. Hum Genomics. 2012;6(1):15. https://doi.org/10.1186/1479-7364-6-15 .
28. Wang Z, Li C, Li X, Shi J, Wu W. Effect of vascular endothelial growth factor rs35569394 in esophageal cancer and response to chemotherapy. Biomol Biomed. 2023;23(2):271-6. https://doi.org/10.17305/bjbms.2021.5891.
29. Bayal AC, Sultana S, Nallari P, Ananthapur V. Genetic polymorphisms of vascular endothelial growth factor (*VEGF*)-2549 I/D and+ 405G/C in the susceptibility to gastric cancer. Arch Clin Gastroenterol. 2021;7(1):001-6.
30. He Y, Ni J, Chen S, Jiang Y, Jia S, Gao Y. The vascular endothelial growth factor-2549 insertion/deletion polymorphism is not associated with susceptibility to hepatocellular carcinoma in Chinese. DNA Cell Biol. 2010;29(7):393-6. https://doi.org/10.1089/dna.2009.1015.
31. Mishra K, Behari A, Kapoor VK, Khan MS, Prakash S, Agrawal S. Vascular endothelial growth factor single-nucleotide polymorphism in gallbladder cancer. J Gastroenterol Hepatol. 2013;28(10):1678-85. https://doi.org/10.1111/jgh.12343.
32. Ungerbäck J, Elander N, Dimberg J, Söderkvist P. Analysis of *VEGF* polymorphisms, tumor expression of *VEGF* mRNA and colorectal cancer susceptibility in a Swedish population. Mol Med Rep. 2009;2(3):435-9. https://doi.org/10.3892/mmr_00000118.
33. Cao YY, Zhang XF, Guo W, Wang R, Ge H, Zhang JH.

Association of the *MDM2* polymorphisms with susceptibility of esophageal squamous cell carcinoma and that of gastric cardiac adenocarcinoma. Tumor. 2007;27(8):628–32.
34. Ma J, Zhang J, Ning T, Chen Z, Xu C. Association of genetic polymorphisms in MDM2, PTEN and P53 with risk of esophageal squamous cell carcinoma. J Hum Genet. 2012;57(4):261-4. https://doi.org/10.1038/jhg.2012.15 .
35. Zhang L, Zhu Z, Wu H, Wang K. Association between SNP309 and del1518 Polymorphism in *MDM2* Homologue and Esophageal Squamous Cell Carcinoma Risk in Chinese Population of Shandong Province. Ann Clin Lab Sci. 2015;45(4):433-7.
36. Cavalcante GC, Amador MA, Ribeiro Dos Santos AM, Carvalho DC, Andrade RB, Pereira EE, et al. Analysis of 12 variants in the development of gastric and colorectal cancers. World J Gastroenterol. 2017;23(48):8533-43. https://doi.org/10.3748/wjg.v23.i48.8533.
37. Dong D, Gao X, Zhu Z, Yu Q, Bian S, Gao Y. A 40-bp insertion/deletion polymorphism in the constitutive promoter of *MDM2* confers risk for hepatocellular carcinoma in a Chinese population. Gene. 2012;497(1):66-70. https://doi.org/10.1016/j.gene.2012.01.004.
38. Gansmo LB, Vatten L, Romundstad P, Hveem K, Ryan BM, Harris CC, et al. Associations between the *MDM2* promoter P1 polymorphism del1518 (rs3730485) and incidence of cancer of the breast, lung, colon and prostate. Oncotarget. 2016;7(19):28637-46. https://doi.org/10.18632/oncotarget.8705.
39. Ahluwalia TS, Khullar M, Ahuja M, Kohli HS, Bhansali A, Mohan V, et al. Common variants of inflammatory cytokine genes are associated with risk of nephropathy in type 2 diabetes among Asian Indians. PLoS One. 2009;4(4):e5168. https://doi.org/10.1371/journal.pone.0005168.
40. Deng N, Zhou H, Fan H, Yuan Y. Single nucleotide polymorphisms and cancer susceptibility. Oncotarget. 2017;8(66):110635-49. https://doi.org/10.18632/oncotarget.22372 .
41. Mandal RK, Agrawal T, Mittal RD. Genetic variants of chemokine CCL2 and chemokine receptor CCR2 genes and risk of prostate cancer. Tumour Biol. 2015;36(1):375-81. https://doi.org/10.1007/s13277-014-2646-x.
42. Singh V, Srivastava P, Srivastava N, Kapoor R, Mittal RD. Association of inflammatory chemokine gene CCL2I/D with bladder cancer risk in North Indian population. Mol Biol Rep. 2012;39(10):9827-34. https://doi.org/10.1007/s11033-012-1849-8.
43. Adeli K, Ogbonna G. Rapid purification of human DNA from whole blood for potential application in clinical chemistry laboratories. Clin Chem. 1990;36(2):261-4.
44. Buraczynska M, Ksiazek P, Baranowicz-Gaszczyk I, Jozwiak L. Association of the *VEGF* gene polymorphism with diabetic retinopathy in type 2 diabetes patients. Nephrol Dial Transplant. 2007;22(3):827-32. https://doi.org/10.1093/ndt/gfl641.
45. Hashemi M, Aminiania S, Ebrahimi M, Simforoosh N, Basiri A, Ziaee SAM, et al. Association between polymorphisms in TP53 and *MDM2* genes and susceptibility to prostate cancer. Oncol Lett. 2017;13(4):2483-9. https://doi.org/10.3892/ol.2017.5739.
46. Raina P, Sikka R, Gupta H, Matharoo K, Bali SK, Singh V, et al. Association of eNOS and *MCP-1* Genetic Variants with Type 2 Diabetes and Diabetic Nephropathy Susceptibility: A Case-Control and Meta-Analysis Study. Biochem Genet. 2021;59(4):966-96. https://doi.org/10.1007/s10528-021-10041-2.
47. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006;22(15):1928-9. https://doi.org/10.1093/bioinformatics/btl268.
48. Oshima Y, Yajima S, Yamazaki K, Matsushita K, Tagawa M, Shimada H. Angiogenesis-related factors are molecular targets for diagnosis and treatment of patients with esophageal carcinoma. Ann Thorac Cardiovasc Surg. 2010;16(6):389-93.
49. Sharma T, Gupta A, Chauhan R, Bhat AA, Nisar S, Hashem S, et al. Cross-talk between the microbiome and chronic inflammation in esophageal cancer: potential driver of oncogenesis. Cancer Metastasis Rev. 2022;41(2):281-99. https://doi.org/10.1007/s10555-022-10026-6.
50. Abdel-Latif MM, Duggan S, Reynolds JV, Kelleher D. Inflammation and esophageal carcinogenesis. Curr Opin Pharmacol. 2009;9(4):396-404. https://doi.org/10.1016/j.coph.2009.06.010.
51. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860-7. https://doi.org/10.1038/nature01322.
52. Boiardi L, Casali B, Nicoli D, Farnetti E, Chen Q, Macchioni P, et al. Vascular endothelial growth factor gene polymorphisms in giant cell arteritis. J Rheumatol. 2003;30(10):2160-4.
53. Chinoy H, Salway F, Fertig N, Tait BD, Oddis CV, Ollier WE, et al. Monocyte chemotactic protein-1 single nucleotide polymorphisms do not confer susceptibility for the development of adult onset polymyositis/dermatomyositis in UK Caucasians. Rheumatology (Oxford). 2007;46(4):604-7. https://doi.org/10.1093/rheumatology/kel359.
54. Intemann CD, Thye T, Förster B, Owusu-Dabo E, Gyapong J, Horstmann RD, et al. MCP1 haplotypes associated with protection from pulmonary tuberculosis. BMC Genet. 2011;12:34. https://doi.org/10.1186/1471-2156-12-34.
55. Zhai R, Liu G, Asomaning K, Su L, Kulke MH, Heist RS, et al. Genetic polymorphisms of *VEGF*, interactions with cigarette smoking exposure and esophageal adenocarcinoma risk. Carcinogenesis. 2008;29(12):2330-4. https://doi.org/10.1093/carcin/bgn210.
56. Rollin J, Payancé A, Gouilleux-Gruart V, Boisdron-Celle M, Azzopardi N, Morel A, et al. Significant effect of *VEGF*A polymorphisms on the clinical outcome of metastatic colorectal cancer patients treated with FOLFIRI-cetuximab. Pharmacogenomics. 2015;16(18):2035-43. https://doi.org/10.2217/pgs.15.139.
57. Abushaqra FM, Al-Mehraby EH, Alyahary JS, Elbager S, Shumo ZA, Mohamed H, et al. Frequency of *MDM2* Promoter Polymorphism del1518 (rs3730485) and its Correlation With Clinic-Haematological Features and Treatment Response in Chronic Lymphocytic Leukemia in Sudanese Patients. Clin Lymphoma Myeloma Leuk. 2023;23:S325.