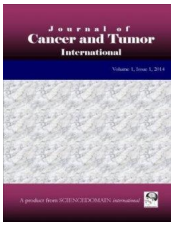

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No Evidence for Association of -2518 A/G Promoter Polymorphism of *MCP-1* with Risk of Esophageal Cancer in Punjab, North-West India

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VS and KG designed the experiments. Authors MS, MM, NRS and MSU did the diagnosis and clinical classification of patients. Author KG performed the experiments. Authors KG and VS analyzed the data, drafted the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the role of *MCP*-1 -2518 A/G polymorphism in the susceptibility to esophageal cancer in patients from Punjab, North-West India.
Methods: In this case-control study, 159 sporadic esophageal cancer patients and 159 age and gender matched controls were included. *MCP*-1 -2518 A/G promoter polymorphism was analyzed using PCR-RFLP method.

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Results: The frequencies of GG, GA, and AA genotypes were 43.40%, 47.80% and 8.80% in patients, and 47.17%, 44.65%, 8.18% among the controls respectively. There were no significant differences in genotype and allele frequencies between the patients and controls.
Conclusion: In this study, we found no association between *MCP*-1 -2518 A/G polymorphism and the esophageal cancer risk in North-West Indians.

Keywords: Esophageal cancer; chemokines; MCP-1; polymorphism.

1. INTRODUCTION

Chronic inflammation has been documented to be associated with the development and progression of different cancers [1,2]. Chemokines, cytokines and transcription factors are main key mediators of cancer related inflammation and they also play an important role in diagnosis, prognosis and treatment of cancers [3-5]. Chemotactic cytokines are small heparin-binding proteins which play a critical role in development, hematopoiesis, lymphocyte trafficking, angiogenesis and cancer [6]. MCP-1 (monocyte chemoattractant protein-1) (OMIM 158105), a member of C**-**C chemokine family, composed of 76 amino acids and is encoded by *CCL2* located at 17q12 [7,8]. MCP-1 alone or along with other cytokine, not only attracts monocytes but can also cause their activation [9] and is also implicated in regulation of cancer cell growth, angiogenesis and metastasis [10-12].

Increased expression of MCP-1 has been observed in many tumors including glioma, esophageal, lung, ovarian, breast and prostate cancer [13,14]. Among gastrointestinal tract tumors, MCP-1 has been considered as a favorable prognostic marker in colon cancer [15-17]. *CCL2* has been described as a crucial mediator of the initiation and progression of chronic colitis-associated colon carcinogenesis. It has been suggested that targeting *CCL2* may be useful in treating colon cancers associated with chronic inflammation [18]. In esophageal squamous cell carcinoma (ESCC), it has been postulated that MCP-1 expression and macrophage infiltration play important roles not only in angiogenesis but also in tumor aggressiveness and may be useful in prediction of clinical outcome in esophagectomized patients. ESCC patients with higher MCP-1 expression had worse five-year survival rate as compared to patients without MCP-1 expression [19].

Single nucleotide polymorphisms (SNPs) have an important role in promoting susceptibility to various diseases as well as the response of the individual to drugs and carcinogens [20]. Functional genetic polymorphisms which alter the regulation of gene expression are predicted to have a significant influence on disease pathogenesis [21]. Polymorphisms in chemokines and their receptors have been reported to be associated with the development of various cancer types [22,23]. Ethnic and population level variations have been reported in the distribution of *MCP*-1 -2518 A/G (rs1024611) promoter polymorphism (24-26). A meta-analysis including 4,162 cases and 5,173 controls reported that *MCP*-1 -2518A/G polymorphism might have some relation to digestive system cancer susceptibility or cancer development in Caucasians [24]. Jia and his colleagues demonstrated that GG genotype of *MCP*-1 -2518A/G polymorphism was associated with decreased risk of cancer in Asians and increased risk in Caucasians [25]. *MCP*-1 -2518 A/G polymorphism has been described as a protective factor for inflammatory bowel disease (IBD) in European populations [26]. It has been demonstrated that G allele of *MCP*-1 -2518 A/G promoter polymorphism was associated with over expression of MCP-1 [27-30]. On the other hand, A allele was associated with upregulation of MCP-1 in Korean Lupus nephritis patients [31]. It has been reported that carriers of A allele have a lower MCP-1 expression and are more susceptible to distant metastasis of nasopharyngeal carcinoma after treatment [32]. ESCC patients with *MCP-1*-2518 GG and *IL-6* -634CG+GG genotype had higher incidence of grade 2-4 platelet count reductions (<75,000/ mm^3) post chemoradiotherapy (CRT) indicating a clinical relevance of polymorphisms for CRT regime [33].

Esophageal cancer (EC) is a common malignant tumor of the digestive tract, ranks eighth in cancer incidence and sixth in cancer mortality worldwide and approximately 80% of cases occur in developing countries [34]. The exact molecular mechanism of EC has not been fully figured out yet, but it is related to several factors including inflammation, genetic factors and lifestyles. The investigations about role of *MCP*-1

-2518 A/G polymorphism in gastrointestinal tract cancers including oral [35,36], gastric cancer [12] and colorectal cancer [37] have yielded conflicting results. The association of *MCP*-1 -2518 A/G polymorphism with EC had not been evaluated yet in India. Therefore, the aim of present case-control study was to investigate the role of *MCP*-1 -2518 A/G polymorphism in the susceptibility to EC in patients from Punjab, North-West India. Understanding the molecular mechanisms involved in esophageal tumorigenesis may be predictive of treatment outcome or development of new treatment modalities. To the best of our knowledge, this is the first study on *MCP-1*-2518 A/G polymorphism in EC from Punjab, North-West India.

2. MATERIALS AND METHODS

2.1 Study Subjects

For this study, patients were clinically investigated at Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar, Punjab. The study group included 159 clinically confirmed esophageal cancer patients and 159 age (± 5 years) and gender matched healthy individuals. The controls were from same geographical region as that of patients. Controls with self reported history of any other cancer were excluded from the study. The characteristics of each subject were collected by interview and from medical records on pre-tested proforma. Five millilitre peripheral venous blood sample of all study participants was collected in EDTA vials and stored at -20°C until use. Experimental procedures were carried out at Human Genetics Department, Guru Nanak Dev University, Amritsar. This study was approved by the Ethics Committee of Guru Nanak Dev University, Amritsar, Punjab, India and written informed consent was obtained from all subjects.

2.2 DNA Extraction and Genotyping

The genomic DNA was extracted from blood using standard phenol chloroform method [38]. The region of DNA harboring *MCP-1* -2518 A/G polymorphism was amplified by using previously published primer sequences [39]. The PCR reaction was set in a total reaction volume of 15 µl, containing 50 ng DNA, 1.5 µl 10X *Taq* buffer with 15 mM $MgCl_2$, 0.4 µl dNTPs mixture (Merck Biosciences), 6 picomole of each primer (Sigma, St. Louis, MO, USA), 1 U *Taq* DNA polymerase (Merck Biosciences). A negative control without template DNA was included in all batches of PCR reaction to monitor the contamination. The PCR conditions were as follows: initial denaturation step at 95°C for 5 minutes followed by 35 cycles with denaturation at 95°C for 45 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and final extension at 72°C for 10 minutes in a Mastercycler gradient, (Eppendorf, Germany). The amplified products were digested with *Pvu*II restriction enzyme (New England Biolabs, Beverly, MA) for overnight at 37°C. The digestion products were resolved on 2.3% ethidium bromide stained agarose gel and visualized under ultraviolet light. The genotype of *MCP-1* -2518 A/G polymorphism was characterized as previously described [40]. Analysis was blindly performed without knowing case/control status. To ensure the genotyping accuracy, ten percent of randomly selected samples were repeated and results were 100% concordant.

2.3 Statistical Analyses

All statistical analyses were carried out using SPSS (Version 16, SPSS Inc, Chicago, IL). Genotype and allele frequencies were calculated by direct counting. The distribution of genotypes in study subjects were examined for deviation from Hardy-Weinberg equilibrium using χ^2 test. Differences in genotype and allele frequencies between cases and controls were estimated by χ^2 test. For estimation of the relative risk and strength of association, odds ratio, their 95% confidence intervals (CI) ranges and corresponding *P* values were calculated using the Web-Assotest program (http://www.ekstroem.com). Genetic models were also used to find the association of polymorphism with the risk of cancer. A *P*-value <0.05 was considered statistically significant.

3. RESULTS

3.1 Characteristics of Study Subjects

The present case-control study comprised 159 esophageal cancer patients and 159 unrelated healthy individuals. Among 159 patients, there were 64 males and 95 females. The mean age was 56.32±13.22 years for cases and 53.82±12.66 years for controls. Squamous cell carcinoma was the predominant tumor type (93.71%) among these patients. Forty eight male and fifty two female patients were diagnosed with cancer after the age 50 years. Male patients with age >50 years had 2.5 times higher risk for EC

as compared to women in the same age group (OR: 2.48, 95%CI: 1.23-4.94; *P*=0.01). Of 159 patients, 24 had stage I, 76 had stage II, 43 had stage III and 16 had stage IV tumors.

3.2 Association between *MCP-1* -2518G/A Polymorphism and Esophageal Cancer Risk

The frequencies of GG, GA, and AA genotypes were 69 (43.40%), 76 (47.80%), and 14 (8.80%), among the cases, and 75 (47.17%), 71 (44.65%), 13 (8.18%) among the controls respectively (Table 1). The distributions of genotypes did not deviate from the Hardy-Weinberg equilibrium in patients (*P* = 0.28) and controls (*P* = 0.50). There were no significant differences in allele and genotype frequencies of *MCP*-1 -2518G/A polymorphism between the patients and controls. All the genetic models exhibited no association with EC. Even on stratifying the subjects according to gender no significant difference in genotype and allele frequencies was observed (Table 2), indicating no association of *MCP*-1 -2518G/A polymorphism with EC, especially ESCC in patients from Punjab, India. In addition, we also stratified our subjects to investigate the relationship of *MCP*-1 -2518 A/G polymorphism with the age, gender, diet, smoking, alcohol drinking, histological type and tumor stage but we did not observe any significant association (*P* >0.05) (data not shown).

Table 1. Distribution of genotype and allele frequencies of *MCP*-1 -2518 A/G polymorphism in esophageal cancer patients and controls

Genotypes/Genetic Models/alleles	Patients n(%)	Controls n(%)	OR	95% CI	*P* value
Total No. of Subjects	159	159	-		-
Total No. of Alleles	318	318			
Genotypes					
AA	69(43.40)	75(47.17)	Reference		
AG	76(47.80)	71(44.65)	1.16	0.73-1.84	0.52
GG	14(8.80)	13(8.18)	1.17	0.51-2.66	0.72
Genetic Models					
Dominant					
(AA vs AG+GG)					
AA	69(43.40)	75(47.17)	Reference		
AG+GG	90(56.60)	84(52.87)	1.16	0.75-1.81	0.50
Over dominant					
(AA+GG vs AG)					
AA+GG	83(52.20)	88(55.35)	Reference		
AG	76(47.80)	71(44.65)	1.13	0.73-1.76	0.57
Recessive					
(AA+AG vs GG)					
AA+AG	145(91.19)	146(91.82)	Reference		
GG	14(8.81)	13(8.18)	1.08	0.49-2.39	0.84
Homozygous codominant					
(AA vs GG)					
AA	69(43.40)	75(47.17)	Reference		
GG	14(8.80)	13(8.18)	1.17	0.51-2.67	0.71
Heterozygous codominant					
(AG vs AA)					
AG	76(47.80)	71(44.65)	Reference		
AA	69(43.40)	75(47.17)	1.16	0.73-1.84	0.52
Alleles					
A	214(67.30)	221(69.50)	Reference		
G	104(32.70)	97(30.50)	1.11	0.79-1.55	0.55

n=Number of subjects; Figures in parentheses represent frequency; OR: odds ratio; CI: confidence interval
HWE: Cases - χ^2 = 1.73, P = 0.28; Controls- χ^2 = 0.450, P = 0.50; Both - χ^2 = 1.624, P = 0.44

Table 2. Genotype distribution and allele frequency of *MCP*-1 -2518 A/G polymorphism in male and females subjects

Genotypes/Genetic Models/alleles	Males (n=64)					Females (n=95)				
	Patients n(%)	Controls n(%)	OR	95% CI	*P* value	Patients n(%)	Controls n(%)	OR	95% CI	*P* value
Genotypes										
AA	24(37.5)	31(48.44)	Reference			45(47.37)	44(46.32)	Reference		
AG	33(51.56)	28(43.75)	1.52	0.73-3.17	0.26	43(45.26)	43(45.26)	0.98	0.54-1.77	0.94
GG	7(10.94)	5(7.81)	1.81	0.51-6.41	0.36	7(7.37)	8(8.42)	0.86	0.29-2.56	0.78
Genetic Models										
Dominant model										
(AA vs AG+GG)										
AA	24(37.5)	31(48.44)	Reference			45(47.37)	44(46.32)	Reference		
AG+GG	40(62.5)	33(51.56)	1.57	0.77-3.17	0.21	50(52.63)	51(53.68)	0.96	0.54-1.69	0.88
Over dominant model										
(AA+GG vs AG)										
AA+GG	31(48.44)	36(56.25)	Reference			52(54.74)	52(54.74)	Reference		
AG	33(51.56)	28(43.75)	1.37	0.68-2.75	0.38	43(45.26)	43(45.26)	1.00	0.57-1.77	1.0
Recessive model										
(AA+AG vs GG)										
AA+AG	57(89.06)	59(92.19)	Reference			88(92.63)	87(91.58)	Reference		
GG	7(10.94)	5(7.81)	1.45	0.44-4.83	0.55	7(7.37)	8(8.42)	0.865	0.30-2.49	0.79
Homozygous codominant										
(AA vs GG)										
AA	24(37.5)	31(48.44)	Reference			45(47.37)	44(46.32)	Reference		
GG	7(10.94)	5(7.81)	1.81	0.51-6.4	0.36	7(7.37)	8(8.42)	0.856	0.29-2.56	0.78
Heterozygous codominant										
(AG vs AA)										
AG	33(51.56)	28(43.75)	Reference			43(45.26)	43(45.26)	Reference		
AA	24(37.5)	31(48.44)	1.52	0.73-3.17	0.26	45(47.37)	44(46.32)	1.02	0.57-1.85	0.94
Alleles										
A	81(63.28)	90(70.31)	Reference			133(70.0)	131(68.95)	Reference		
G	47(36.72)	38(29.69)	1.37	0.82-2.32	0.23	57(30.0)	59(31.05)	0.952	0.62-1.47	0.82

n=Number of subjects; Figures in parentheses represent frequency; OR: odds ratio; CI: confidence interval

4. DISCUSSION

Genetic variants in chemokine and chemokine receptor genes have been reported to alter the protein expression which plays a critical role in development of different types of cancer [27,41]. In some gastrointestinal tract cancers, *MCP*-1 -2518A/G has been linked to cancer risk [12,36]. *MCP*-1 -2518A/G polymorphism has been linked to cancer susceptibility especially to digestive tract cancers in Caucasians [24]. The GG genotype appears to increase cancer risk in Caucasians and decreases risk in Asians [25]. In the present study, the subjects were of mixed Caucasian and Indoscythian racial stock [42] inhabiting state of Punjab in North-West part of India. About 8% of both patients and controls had GG genotype. More than 40% of subjects had AA and AG genotypes (Table 1). The AA genotype of *MCP*-1 -2518A/G polymorphism has been linked to lower expression of MCP-1 and an increased susceptibility to distant metastasis in nasopharyngeal carcinoma after treatment [32]. In the present study, we found no evidence for association between this polymorphism and EC risk. Similar results have been reported in some previous studies from India where no association of *MCP*-1 -2518 A/G polymorphism was observed in bladder [43] and prostate [44] cancer. GG genotype of *MCP*-1 -2518 A/G polymorphism was associated with increased risk for gastric cancer in Chinese [12], oral cancer in Turkish [36] and IBD in Polish [45] population.

The results of present study were in contrast to our previous report on Breast cancer in subjects from same geographical area and same racial stock [40]. The GG genotype and G allele was associated with increased risk for breast cancer. Majority of the patients were having infiltrating ductal carcinoma (IDC). The difference in the results is probably due to different etiology of ESCC and IDC.

Majority of the patients (79.25%) in the present study belonged to the rural areas and 93.71% of them had ESCC. In patients, 13.21% subjects were smokers and 31.45% were alcoholic. The etiology and epidemiology of esophageal adenocarcinoma (EAC) and ESCC are greatly different. Chronic inflammation of esophageal lining, chronic reflux, esophagitis and Barrett's esophagus are the main risk factors for EAC, [46,47]. For ESCC, smoking, alcohol consumption and lower socioeconomic status are a few risk factors [47-51]. ESCC is most common in South-eastern and Central Asia accounting about 79% of total ESCC cases worldwide [52].

In the present study, the number of female patients (59.75%) was higher as compared to male patients (40.25%). The gender differences might be due to local conditions like dietary patterns. Majority of the females were anemic, consumed carbohydrate or fat rich but micronutrient deficient diet. In overall cases, 62.89% of EC patients were of age >50 years. Male patients with age >50 years had 2.5 times higher risk for EC as compared to female patients. It was similar to previous report that male patients in the age group between 50-70 years had 3-4 times higher risk for EC as compared to women in the same age group [53-55]**.** Aging has been described as one of the strongest predictors of clinical outcomes in chronic human diseases such as type 2 diabetes, cardiovascular disorders, rheumatoid arthritis, neurodegenerative diseases and cancer because of immunosenescence associated with inflammaging [56-60].

The discrepancy in the results reported by different studies might be due to various factors such as study design, sample size, geographical locations, ethnicity and genetic factors that predispose to various cancers. Meta-analysis involving 3137 individuals (1818 IBD cases and 1319 controls) documented that *MCP*-1 -2518A/G polymorphism might be a protective factor for IBD in European but not in Asian and African patients [26]. The GG and GA genotypes have been reported to be associated with increased risk of acute pancreatitis in Chinese patients [61]. A meta-analysis conducted by Fang and his colleagues depicted that G allele was associated with risk for severe acute pancreatitis [62]. *MCP-1* -2518 A/G polymorphism was not associated with colorectal cancer in Spanish [37] and hepatocellular carcinoma in Taiwanese [63] patients. A meta-analysis of 13 case-control studies involving a total of 2525 cases and 3243 controls also revealed no association between *MCP*-1 -2518 A/G polymorphism and increased cancer risk [64].

5. CONCLUSION

In this study, we found no association between *MCP*-1 -2518 A/G polymorphism and the EC risk in North-West Indians. Though, majority of subjects had AA or AG genotype, of which AA has been previously linked to lower MCP-1

expression and increased susceptibility to metastasis [32]. In future, a comprehensive study on *MCP*-1 polymorphisms along with its expression is required to understand the exact role of this gene in the pathogenesis of EC.

CONSENT

Written informed consent was obtained from all subjects.

ETHICAL APPROVAL

This study was approved by the Ethics Committee of Guru Nanak Dev University, Amritsar, Punjab, India.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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