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Original Research

Relationship between MCP1 (-2518A>G) gene variants and ovarian cancer in Turkish population

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Abstract: The monocyte chemoattractant protein-1 (MCP-1) gene polymorphism(-2518A>G) in the regulatory region of the MCP-1 protein has been reported to be associated with cancer risk. In this study we aimed to investigate the relationship of MCP-1 (-2518A>G) gene polymorphism and ovarian cancer. MCP-1 genotyping was performed using polymerase chain reaction from blood samples ofovarian cancer patient (n=56) and a control groups (n=52).There was a significant difference in MCP1 (-2518A>G) genotypes between the patient and control groups (p=0.049; x^2=6.042). AA carriers were significantly higher in the control group (p=0.014) whereas AG genotype and G allele carriers were significantly higher in the ovarian cancer group (p=0.029, p=0.014, respectively). This study suggests that MCP-1 (-2518A>G) AG genotype and G allele could be considered as risk factor for susceptibility to ovarian cancer.

Key words: MCP1 (-2518A>G); Single nucleotide polymorphism; Genetic variability; Case-control study; Ovarian cancer.

Introduction

Ovarian cancer is the one of the most common gynecological cancer with high mortality in women. Unfortunately, most of the patients are diagnosed at advanced stages of ovarian cancer and it is adisease affecting more than 190,000 women worldwide each year(1). Inflammatory chemokine expression levels are related with some important diseases including cancer (2). It has been shown that several chemokines has emerging role in progression and development of ovarian cancer (3).

Chemokine-receptor interactions are known to be related to many biological processes such as angiogenesis, regulation of cell proliferation, tumor growth and metastasis. Monocyte chemo-attractant protein-1 (MCP-1), also named as chemokine (C-C motif) ligand 2 (CCL2), is a proinflammatory mediator chemokine and encoded by CCL2 gene locating on 17q11.2-q12 (5). It activates macrophagesto infiltrate the tumors and plays crutial role in the host immune response against solid carcinomas. MCP-1 is a key chemokine to induce the recruitment of macrophages in human tumors(6). MCP-1 was thought to have affects on accumulation of tumor-associated macrophages that may influence ovarian tumor behaviors (4, 7, 8). MCP1 (-2518A>G) gene polymorphism was found to belocated in the regulatory region of CCL2 gene and has particulary affect on expression of MCP-1 (9).

In the present case-control study, we investigated the relationship of MCP1 (-2518A>G) gene polymorphism and ovarian cancer in a Turkish population.

Materials and Methods

Study group

A total of 108 women who admitted the Gynecology Clinics of Yeditepe University Hospital , Department of Obstetrics and Gynecology were included in the study. Healthy control group were selected within the women admitting for gyneocological examination within routine check-ups. The diagnosis of ovarian cancer was established by histopathological examination. Ovarian cancer staging was performed due to the pathological FIGO classification revised in 2014. Local Ethical Comittee approval was obtained for the study. The followed protocol was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

DNA extraction

Blood samples from all participants were collected in tubes containing ethylene diamine tetra acetic acid (EDTA). Nuclear DNA was extracted from 350 µl peripheral whole blood using Invitrogen iPrep PureLink gDNA blood isolation kit with a iPrep Purification Instrument (Invitrogen, Life Technologies, Carlsbad, California, USA). The isolation procudure was performed in a closed system and took 45 min; 100 µl of DNA was obtained at the end of the procedure. Consequently, sample DNA concentrations and optical density ratios (at 260/280 nm) were measured by Nanodrop 2000

(Thermoscientific, Waltham, Massachusetts, USA). Isolated DNA samples were preserved at 4°C until genotyping assessments were conducted.

Genotyping

Polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP) analysis was performed for the detection of the variations in MCP1 (-2518A>G) (rs1024611) gene and restriction fragment length polymorphism, the procedures of PCR-RFLP are given in Table 1 and an example of the gel analysis is shown in Figure 1. PCR was initially performed to determine the polymorphic regions using suitable primers. PCR products of MCP1 (-2518A>G)were further subjected to digestion with PvuII restriction enzyme.

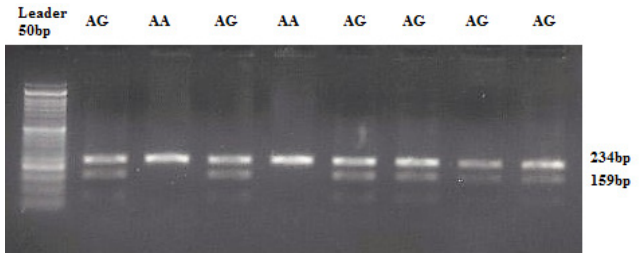

Figure 1. An example of the gel picture of PCR-RFLP analysis results.

The digested products were analyzed on 2% agarose gels stained with ethidium bromide and examined under transillumination.The relative size of the PCR products were determined through comparison of the migration of a 50bp DNA molecular weight ladder (Invitrogen, Grand Island, NY, USA) (10) (Figure 1). All genotypes were read by 2 independent researchers. In any conflicts, the procedures were repeated.

Statistical analyses

Statistical analyses were performed using SPSS version 23 software (SPSS Inc, Chicago, IL, USA). Values were given as the mean ± standard deviation (SD). Student's t-test was used to examine the significance of differences between the two groups by usingPearson Chi-square and Fisher's exact tests to compare demographic informations and determine the effect of each genotype on the risk of ovarian cancer. The relative risk at a 95% confidence interval (CI) was calculated as the odds ratio (OR) and p-values lower than 0.05 were denoted as statistical significance.

Results

Demografic characteristics of patients with ovarian cancer and controls were shown in Table. There was

Table 1. PCR and RFLP procedure and expected products of MCP1 (-2518A>G) gene.

Primers (forward and reverse)	**5' – TCT CTC ACG CCA GCA CTG ACC – 3'** **5' – GAG TGT TCA CAT AGG CTT CTG – 3'**
PCR conditions	25µl of PCR mixture: 1 Mm of each dNTP, 10 pmol/ µl of each primer, 25 mM of MgCl2, 1U Taq polymerase 35 cycles; 95 ◦C 45 s, 52 ◦C 45 s, 72 ◦C 45 s
PCR product	234 bp
Restriction enzyme	PvuII
Restriction products	AA: 234 bp AG: 234 bp, 159 bp, 75 bp GG: 159 bp, 75 bp

Table 2. Demographic characteristics of the study population.

Parameter			**Ovarian Cancer (n=56)**	**Control (n=52)**	***p*-Value**
Age (years), mean±SD			57.38±10.03	61.02±11.49	0.081
Height (cm), mean±SD			159.25±6.09	160.67±5.03	0.213
Weight (kg), mean±SD			73.84±14.47	73.67±10.79	0.947
Body mass index (kg/m), mean±SD			29.46±6.17	28.64±4.76	0.459
Smoking	Yes		16.4%	17.3%	0.896
	No		83.6%	82.7%	-
Hypertension	Yes		56.6%	19.2%	<0.001*
	No		43.4%	80.8%	-
Diabetes	Yes		24.5%	9.6%	0.043*
	No		75.5%	90.4%	-
Pathological FIGO stage, n (%)		I-II	58.3%	-	-
		III-IV	41.7%	-	-
Metastasis		Yes	78.4%	-	-
		No	21.6%	-	-
Relapse		Yes	50%	-	-
		No	50%	-	-

n: number of individuals; SD: standard deviation;*statistically significant difference.

Table 3. MCP1 (-2518A>G) genotype and allelle frequencies in patients with ovarian cancer and the control group.

	Ovarian Cancer (n=56)	**Control (n=52)**	***p*-Value**	***odds ratio (OR)***	***Chi Squre***	***95% confidence interval (CI)***
Genotype	n (%)	n (%)				
AA	28 (50%)	38 (73.1%)	0.014*	0.368	6.042	0.165-0.825
AG	24 (42.9%)	12 (23.1%)	0.029*	2.500	4.747	1.085-5.759
GG	4 (7.1%)	2 (3.8%)	0.455	1.923	0.559	0.337-10.970
AG+GG	28 (50%)	14 (26.9%)	0.014*	2.714	6.042	1.212-6.078
Allelle	Allelic count (%)	Allelic count (%)				
A	80 (71.4%)	88 (84.6%)	0.455	0.520	0.559	0.091-2.966
G	32 (28.6%)	16 (15.4%)	0.014*	2.714	6.042	1.212-6.078

n: Number of individuals; *statistically significant difference.

no statistical significance between the ages of patient and control groups, mean of agewere 57.38±10.03and 61.02±11.49, respectively (p=0.081). Sysytolic hypertension and diabetes were significantly frequent in ovarian cancer group compared to the controlcases (p<0.001, p=0.043).

Table 3 shows the frequency of MCP1 (-2518A>G) and their respective alleles among cases and controls. There was a significant difference in MCP1 (-2518A>G) genotypes between patient and control groups (x^2=6.042; p=0.049). MCP1 (-2518A>G) AA, AG and GG genotype frequencies for controls were 73.1%, 23.1% and 3.8% and ovarian cancer genotype frequencies were 50%,42.9% and 7.1%, respectively. Individuals with AG genotype had 2.5-fold risk of developing ovarian cancer(p=0.029, x^2=4.747, OR=2.5, 95%CI=1.085-5.759). AG+GG genotype carriers also had 2.7 fold increased ovarian cancer risk compared to control group (p=0.014). In contrary,AA genotype carriers had a 2.7-fold decreased riskof ovarian cancer (p=0.014, x^2= 6.042, OR=0.368, 95%CI= 0.165-0.825). The frequency of G allele was significantly higher in the patient group (p=0.014, x^2= 6.042, OR=2.7, 95%CI= 1212-6.078) while A allele frequency was not statistically significant (p=0.455, x^2= 0.559, OR=0.5, 95%CI= 0.091-2.966).

Discussion

In the current study, we investigated the relationshipbetween MCP1 (-2518A>G) polymorphism and susceptibility to ovarian cancer in a Turkish population.

The monocyte chemoattractant protein-1 (MCP-1) shows its biologic effects via binding to a G-protein coupled receptor (CC chemokine receptor). Hence, MCP-1have roles in regulating the monocytes and increasingcellular migrations in cancer related inflammation, angiogenesis and metastasis (11).Increased levels of MCP-1was observed in various tumorsandelevated MCP-1 levels was shown to be associated with angiogenesis and tumor cell proliferation (12). MCP-1 mediates tumor proliferation and migration via phosphatidylinositol 3-kinase/protein kinase B-dependent signaling (13).Weiet al (2015) demonstrated that AG and GG genotype carriers for gene region MCP-1 (-2518A>G) had higher serum levels of MCP-1 than homozygote wild type carriers. Thereby they suggested that mutant allele carriers had increased susceptibility to ovarian cancer. Moreover, they found that MCP-1 serum levels were significantly higher in AG and GG genotype carriers (14).

In our study, there was a 2.5-fold cancer risk increment inAG genotype individuals. Moreover, G allele carriers were significantly higher in the patient group (p=0.014), thus carrying G allele was considered to be a risk factor for ovarian cancer.It has been shown that G allele had higher activity than wild type A allele on the gene promoter sites (15). This data replicated in cell lines by Pham et al (2012) (16). They indicated that cells with GG genotypes had increased transcription activity and had more capacity to synthesize MCP-1 protein than AA homozygote cells (16).Immunohistochemistry analysis indicated that MCP-1 expression levels were higher in AG and GG genotype carriers than individuals having AA genotype (17). In addition to that, association of increased serum MCP-1 levels and MCP1 (-2518A>G) gene polymorphism was also demonstrated in patients with nasopharyngeal carcinoma (18). Those data corfirmed our results that AG+GG genotype carriers had 2.7 fold ovarian cancer risk rather than control group.

In the current study, we determined the relationship between MCP1 (-2518A>G) gene polymorphism and ovarian cancer. MCP1 (-2518A>G) AA carriers were significantly higher in the control group (p=0.014) whereas AG genotype and G allele carriers were significantly higher in the ovarian cancer group. Despite limitations, the changes MCP-1 gene region could be considered as risk factor for susceptibility to ovarian cancer. Further investigationswith larger study groups areneeded to gain additional insight to the mechanisms which could be related to MCP-1 polymorphisms.

Interest conflict

The authors declare no potential conflicts of interest relevant to this article.

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