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LOW LEVEL OF MATRIX METALLOPROTEINASE-1 IN PATIENTS WITH BLADDER CANCER AND THE EFFECT OF MMP-1 POLYMORPHISM (-1607) ON SERUM MMP-1 LEVELS AND THE RISK OF BLADDER CANCER

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Abstract – *Objective:* *Matrix metalloproteinases play an important role in the development and progression of cancerous tumors. The current study aimed at determining the genetic polymorphism in matrix metalloproteinase-1 (MMP-1) gene (rs1799750) and susceptibility to bladder cancer, evaluating serum MMP-1 level, and investigating the effect of the polymorphism on serum MMP-1 levels.*

Materials and Methods: *A total of 157 patients with bladder cancer and 143 healthy subjects were recruited. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was employed to determine genetic polymorphism. Serum MMP-1 level was measured by the enzyme-linked immunosorbent assay (ELISA) technique.*

Results: *The mean serum level of MMP-1 was 2.676 ±1.77 ng/mL in the patient group and 3.00 ±1.69 ng/mL in the control group. There was a significant decrease in serum MMP-1 level in the patient group compared with the control group. There was no significant association between MMP-1 polymorphism and susceptibility to bladder cancer. A three-fold higher risk of bladder cancer was associated with smoking.*

Conclusions: *The genetic variation in MMP-1 (–1607) polymorphism is not associated with the risk of bladder cancer. It seems that serum MMP-1 levels of genetic variants are not related to the MMP-1 (–1607) polymorphism.*

KEYWORDS: *Bladder cancer, Serum levels of MMP-1, MMP–1 Polymorphism (–1607).*

INTRODUCTION

Bladder cancer (BC) is the 11th most prevalent type of cancer by incidence and ranks 14th by the associated mortality in the world[1]. The incidence and mortality rate of BC increases mainly due to the changes in the risk factors. The highest incidence of bladder cancer is reported in Egypt, Europe, and South and North Africa[2], while the Asian countries have the lowest incidence [3]. In addition, it is the second most common tumor of the genitourinary tract after prostate cancer[4]. It is approximately four-fold more frequent in males and more common in white people. The most important risk factors of BC, as reported in different studies, include smoking and exposure to urothelial carcinogens. Smoking increases the risk of BC by two to four times[5]. Matrix metalloproteinases (MMPs) interventions at different stages provide a possible prognosis for a cancer patient[6]. Recent studies show an increased fibroblastic collagenase (MMP-1) expression in various tumor tissues. This enzyme is expressed by tumor stem cells and fibroblasts, which imposes physical barriers on the progression and invasion of cancerous cells by damaging the base membrane and extracellular matrix. Finally, by releasing factors such as insulin-like growth factor (IGF), basic fibroblast growth factor (BFGF), and vascular endothelial growth factor (VEGF), it provides a favorable condition for tumor growth[7-10]. The expression of *MMP-1* and its function in transplant digestion can be affected by genetic polymorphism in the promoter region of the *MMP-1* gene. The addition of a guanine base leads to a sequence that causes continuous activation of ETS transcription factors (5`-GGAT-3`). This position lies in the vicinity of the AP-1 connection site at the –1607 position. The ETS

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family requires other transcription factors such as the AP-1 family to induce transcription. The two adjacent sites act synergistically and together increase the transcription of the 2G allele. As a result, the 2G/2G genotype can increase the *MMP-1* gene expression, which potentiates and facilitates the spread of metastasis and relapse and recrudescence of the disease in patients with cancer[11,12]. The association of *MMP-1* polymorphism with cancers including renal cell carcinoma, ovary tumor, colorectal cancer, lung cancer, and head and neck cancer is well documented[13-17]. According to these studies, tumor cells and different tissue express different amounts of fibroblastic collagenase. Hence, it is possible that the enzyme plays a major role in spreading and metastasis of a particular type of cancer in a particular population, while it has no significant role in a different population and type of cancer. The current study aimed at detecting the promoter polymorphism of *MMP-1* (–1607) 1G/2G (rs1799750) and the risk of BC and investigating the effect of the genetic polymorphism on serum MMP-1 level, which is not reported in Iran yet.

MATERIALS AND METHODS

Sample collection

In the current case-control study, blood and serum samples were collected from 157 patients with BC (aged 60.89 ±13.62 years) and 143 healthy subjects (aged 60.4 ±16.44 years) as a control group. All bladder cancer samples were transitional cell carcinoma (TCC). The classification and determination of the stage and grade were conducted based on the report of hospital pathologists. The tumors were classified into four stages of Ta, T1, T2, and T3. The tumors were classified into two categories of low and high grades. The clinicopathological data are summarized in Table 1. The subjects of the control group were referred to the hospital for an annual checkup and did not have any cancers. After coordination with the personnel and physicians of Hashemi-Nejad Hospital operating room and receiving written consent from the individuals, the samples were collected from July 2014 to August 2016. After collecting samples from the subjects, blood separation from serum was performed and the specimens were frozen at -20°C. The current study was approved by the Ethics Committee of Islamic Azad University and was performed based on the principles of Helsinki Declaration.

DNA extraction, PCR-RFLP amplification, and ELISA technique

DNA extraction was performed using a DNA isolation kit for mammalian blood from Roach Life Science (Cat. No. 11 667 327 001, Mannheim, Germany) following the manufacturer's instructions, and the samples were stored at -20°C.

TABLE 1. The clinical and clinicopathological characteristics of the study subjects.

Sample Characteristics	Number of Samples	
	Patient Group N=157	Control Group N=143
Sex		
Male	129 (82.17%)	121 (84.62%)
Female	28 (17.83%)	22 (15.38%)
Smoking		
Yes	89 (56.69%)	41 (28.67%)
No	68 (43.31%)	102 (71.33%)
Tumor Stage		
Ta	92 (58.6%)	
T1	48 (30.57%)	
T2	16 (10.19%)	
T3	1 (0.64%)	
Tumor Grade		
Low	72 (45.86%)	
High	85 (54.14%)	

The PCR was optimized in a final volume of 25 µL containing 12.5 µL amplicon master mix buffer, 50 ng DNA, and 0.5 µL (0.4 µM) of *MMP-1* specific primers (F: 5'-TGACTTTTAAAACATAGTCTATGTTCA-3', R: 5'-CTTGGATTGATTTGAGATAAGTCATAGC-3'). PCR was performed using a BioRad PCR system (BioRad, Hercules, CA, USA) with the following program: initial denaturation at 95°C for 3 min, 35 cycles at 95°C for 30 s, 55°C for 40 s, and 72°C for 1 min, and a final extension at 72°C for 5 min. The electrophoresis of the PCR products (Bio-Rad System, Hercules, CA, USA) after staining with DNA safe stain on 2% agarose gel was investigated and the gels were visualized using a Gel Doc device (Uvitech, UK).

In order to optimize the RFLP reaction, 10 µg DNA, 2 µL 10X TangoTM buffer (Fermentas, Thermo Scientific, Waltham, MA, USA), 1 µL restriction enzyme *Alu*I (Fermentas, Thermo Scientific, Waltham, MA, USA), and 6 µL of the PCR product were used. The samples were incubated at 37°C for 16 hours for the reaction of *Alu*I restriction enzyme and for 20 min at 65°C to deactivate the enzyme. This enzyme cuts at 1G allele, the site of polymorphism. After electrophoresis, genotypes 1G/1G with two bands of 241 and 28 bp, 2G/2G genotypes with a single band of 269 bp, and heterozygotes with a combination of all three bands were recognized. Serum MMP-1 level was measured by an MMP-1 kit (EAST BIOPHARM) according to the manufacturer's instructions by enzyme-linked immunosorbent assay (ELISA) technique (TECAN-Sunrise, USA-ELISA reader).

Statistical Analysis

The association between *MMP-1* polymorphism (–1607) and the risk of BC were investigated using chi-square test. Adjusted odds ratio (OR) was calculated for age and smoking, by logistic regression model when cancer was used as a dependent variable and genotype was taken as independent variable. The correlation between serum *MMP-1* levels, age, and risk of cancer was determined by Spearman correlation test. The association between serum MMP-1 levels, age, and risk of cancer were analyzed by logistic regression analysis (logistic regression analysis where cancer was used as a dependent variable, and MMP-1 and age, were taken as independent variables). The correlation between MMP-1 serum level and age was analyzed by Pearson correlation. The correlations between MMP-1 serum levels and gender, smoking, and the other categorical traits were determined by Spearman correlation. The serum level of MMP-1 was expressed as mean ± standard deviation (SD) and median in groups using the Mann-Whitney U-test. The association between polymorphism and bladder cancer was determined by GraphPad Prism software (version 6.01 La Jolla, CA, USA). The Hardy-Weinberg equilibrium was calculated using POPGENE software (version 1.32). The correlation and logistic regression as well as adjusted OR were analyzed by MedCalc software (version 15.8). In all calculations, $p<0.05$ was considered statistically significant and the confidence interval (CI) and OR were measured.

RESULTS

Clinical and Clinicopathological characteristics of the subjects

A total of 157 patients with the mean age of 60.89±13.62 years and 143 healthy subjects with the mean age of 60.40±16.41 years were recruited by the study. The clinical and clinicopathological characteristics of the participants are presented in Table 1. Smoking was considered as a risk factor for BC in the two groups of patients and controls. Eighty-nine patients with cancer and 41 individuals in the control group were smokers. There was a significant association between smoking and the susceptibility to BC. According to the OR number, a three-fold higher risk of BC was observed for smokers (OR: 3.25, 95% CI: 2.01–5.26; $p = 0.0001$).

Genotyping and polymorphism analysis

The results of PCR-RFLP on the 2% agarose gel are shown in Figure 1. Allele and genotype frequencies for both patient and healthy subjects are shown in Table 2. No significant difference in the allele frequency was observed ($p = 0.37$). The healthy and patient groups were in equilibrium ($p = 0.66$) and no association was observed between the genotypes and the risk of BC (Table 3). As shown in Table 4, there was no significant association between the 2G/2G genotypes and smoking ($p > 0.05$) (OR: 0.7, 95% CI: 0.23–2.1; $p = 0.52$). Tumors were classified into three genetic groups and two clusters based on size (Table 4). There was no significant association between the genotypes and tumor size ($p > 0.05$).

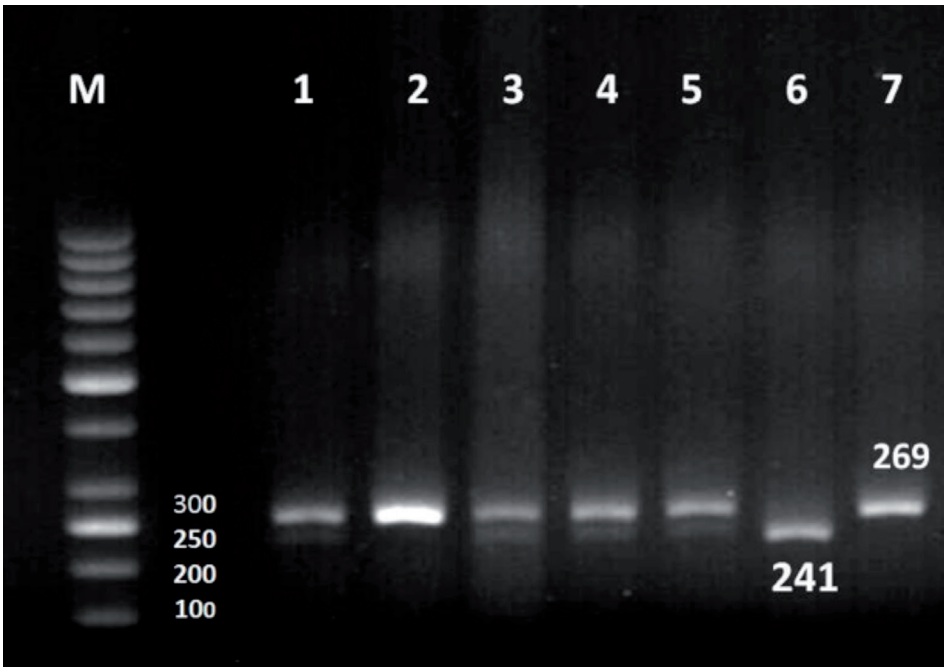

Fig. 1. Electrophoresis patterns from *AluI* digestion to identify polymorphisms MMP-1 (–1607). Lines 2,7: 2G/2G genotype (269 bp); lines 1,3,4,5: 1G/2G genotype (269 bp, 241 bp); line 6: 1G/1G genotype (241 bp); M: a 50-bp DNA ladder.

TABLE 2. Genotypic and genetic frequency and hardy-weinberg equilibrium.

Genotype	Case (%) N = 157	HWE of Case p-value[a]	Control (%) N = 143	HWE of control p-value	Genotypic p-value[b]
1G/1G	30 (21.00)	0.001*	30 (19.10)	0.009*	0.66
1G/2G	54 (34.40)	(χ^2 = 10.58)	54 (37.76)	(χ^2 = 6.67)	
2G/2G	73 (46.50)		59 (41.25)		

HWE: Hardy-Weinberg equilibrium. Significant level;* = $p < 0.05$.
[a]Based on the results of χ^2-test.
[b]Based on the results of χ^2-test for the comparison between patients and control groups.

Forty patients with 2G/2G genotype and 33 patients with 2G/2G genotype were high grade and low grade, respectively. But no significant association was observed between the genotypes and grade of disease (Table 4). It seems that there was no significant association between the genotypes and stage of the disease (Table 4).

The association of smoking with the disease grade, the size of the tumor, and the stage of the disease

The smoking habit was analyzed on the basis of the grade of cancer. Thirty-four patients among the non-smokers had low-grade BC, while 34 patients were with high-grade. Fifty-one patients among the smokers had high-grade BC and 38 patients had low-grade BC (Table 5). There was no significant association between disease grade and smoking (p = 0.36). There was no significant association between smoking and the stage of the disease (p = 0.49) and tumor size (p = 0.15) (Table 5).

MMP-1 Serum Level

MMP-1 serum level in the patient and control groups was 2.676 ±1.77 and 3.00 ±1.69 ng/mL, respectively. The MMP-1 level was higher in the control group than the patient group, which the difference between the groups was statistically significant (p = 0.005).

The association and correlation between serum level of MMP-1, age, and incidence of cancer are illustrated in Table 6. A direct significant association was observed between age and serum level in such a way that the serum levels of MMP-1 increased with age. However, there was no association and correlation between the serum MMP-1 level and the incidence of cancer (p >0.05). There was no significant correlation between serum MMP-1 levels and smoking in all samples (p >0.05). However, there was no significant association between serum level and male gender (p = 0.058). The correlations between serum level of MMP-1 in the control group with age, gender, and smoking were calculated separately. There was no significant correlation between MMP-1 serum level and age, gender, and smoking in the control group (p >0.05). No correlation was observed between MMP-1 serum level and age, gender, smoking, grade and stage of disease, and tumor size in patients (p >0.05) (Table 7).

The mean serum level of 1G/1G, 2G/2G, and 1G/2G genotypes were 3.148 ±1.88, 3.13 ±2.08, and

TABLE 3. Odds ratios for the association between the genetic variants of MMP-1 (–1607) and susceptibility to BC.

Genotype	Case (%) N = 143	Control (%) N = 157	OR (95% CI)[a]	p-value[a]	OR Adjusted[b] (95% CI)	p-value[b]
1G/1G	30 (19.10)	30 (21.00)	1 (Reference)		1 (Reference)	0.38
1G/2G	54 (34.40)	54 (37.76)	1.00 (0.53 – 1.9)	1.00	0.61 (0.2 – 1.8)	0.46
2G/2G	73 (46.50)	59 (41.25)	1.23 (0.67 – 2.28)	0.49	0.82 (0.48 – 1.4)	0.38
1G/2G+2G/2G	127 (80.00)	113 (79.02)	1.12 (0.64 – 1.98)	0.68	0.64 (0.23 – 1.7)	
Recessive model						
1G/2G+1G/1G	84 (53.50)	84 (58.75)	1 (Reference)		1 (Reference)	
2G/2G	73 (46.50)	59 (41.25)	1.23 (0.78 – 1.95)	0.36	0.93 (0.44 – 1.9)	0.86
Allele						
1G	114 (36.30)	114 (39.86)	1 (Reference)			*p*-value
2G	200 (63.70)	172 (60.14)	1.16 (0.83 – 1.6)			0.37

[a]OR was analyzed by χ^2-test.
[b]Logistic regression analysis where cancer was used as a dependent variable, and genotype was taken as independent variable. Model adjusted for smoking and age.
CI = confidence interval; OR = Odds Ratio; BC: bladder cancer.

TABLE 4. The association of MMP-1 (–1607) genotypes with smoking, tumor size, grade, and stage of cancer.

		Smoking Status		
Genotypes				
Never Smokers	***Case (%), N=68***	***Control (%), N=102***	***OR (95% CI)***	***p-value***
1G/1G	12 (17.64)	24 (23.52)	1 (Ref)	
1G/2G	21 (30.88)	37 (36.27)	1.13 (0.47-2.7)	0.77
2G/2G	35 (51.47)	41 (40.19)	1.70 (0.74-3.9	0.20
1G/2G+2G/2G	56 (82.35)	78 (76.47)	1.43 (0.66-3.1)	0.35
Ever Smokers	***Case (%), N=89***	***Control (%), N=41***	***OR (95% CI)***	***p-value***
1G/1G	18 (20.22)	6 (14.63)	1 (Ref)	
1G/2G	33 (37.07)	17 (41.46)	0.64 (0.21-1.9)	0.43
2G/2G	38 (42.70)	18 (44.00)	0.7 (0.23-2.1)	0.52
1G/2G+2G/2G	71 (79.77)	35 (85.36)	0.67 (0.24-1.8)	0.44
Tumor Size	***Tumor Size ≥3 cm Case (%), N=77***	***Tumor Size <3 cm Case (%), N=80***	***OR (95% CI)***	***p-value***
1G/1G	18 (23.37)	12 (15.00)		
1G/2G	27 (35.06)	27 (33.75)	0.66 (0.27-1.6)	0.37
2G/2G	32 (41.55)	41 (51.25)	0.52 (0.22-1.2)	0.13
1G/2G+2G/2G	59 (76.62)	68 (85.00)	0.57 (0.25-1.3)	0.18
Grade	***High Case (%), N=85***	***Low Case (%), N=72***	***OR (95% CI)***	***p-value***
1G/1G	15 (17.64)	15 (20.83)	1 (Ref)	
1G/2G	30 (35.29)	24 (33.33)	1.25 (0.51-3.0)	0.62
2G/2G	40 (47.05)	33 (45.83)	1.20 (0.51-2.8)	0.65
1G/2G+2G/2G	70 (82.35)	57 (79.16)	1.20 (0.5-2.7)	0.61
Stage	***T2 and T3 Case (%), N=17***	***Ta and T1 Case (%), N =140***	***OR (95% CI)***	***p-value***
1G/1G	5 (29.41)	25 (17.85)	1 (Ref)	
1G/2G	8 (47.05)	46 (32.85)	0.87 (0.25-2.9)	0.82
2G/2G	4 (23.52)	69 (49.28)	0.29 (0.07-1.1)	0.06
1G/2G+2G/2G	12 (70.58)	115 (82.14)	0.52 (0.17-1.6)	0.25

CI = confidence interval; OR = Odds Ratio.

2.70 ±0.86 ng/mL in the control group and 2.979 ±2.44, 2.410 ±1.27 and 2.632 ±1.35 ng/mL in the patients group, respectively. No significant association was observed between MMP-1 serum level and genotype of subjects (p >0.05).

The average MMP-1 serum level was evaluated separately in the three genotypes and the results are presented in Table 8. In all groups, MMP-1 levels were higher in the controls, but there was no significant association between the genotypes of the healthy and patient groups (p >0.05).

DISCUSSION

According to the suggested role of MMP-1 in cancer, the effect of *MMP-1* (–1607) polymorphism and its association with the serum MMP-1 level and the risk of BC was investigated in the current study. In the current study, the three-fold risk of BC by smoking was observed in smokers. No association was observed between the allele frequency and the risk of BC. There was no association between the gene polymorphism and the risk of BC. The mean serum levels in the subjects with BC were lower than those of the control group. The mean serum levels associated with the three genotypes were measured in the control and patient groups and no statistically significant association was noted between them. The association of serum MMP-1 level with age, gender, and smoking were studied separately in the healthy and patient samples and no significant association was found between the male gender and the serum level. There was no significant association

TABLE 5. Odds ratios for the association between the genetic variants of MMP-1 (–1607) and susceptibility to BC.

Cancer Grade	*High Grade (%) N (%)*	*Low Grade (%) N (%)*	*OR (% 95 CI)*	*p-value*
No Smoking	34 (50.00)	34 (50.00)	1 (Reference)	0.36
Smoking	51 (57.30)	38 (42.70)	1.34 (0.71-2.5)	
Tumor Size	*Tumor Size ≥ 3 cm N (%)*	*Tumor Size < 3 cm N (%)*	*OR (% 95 CI)*	*p-value*
No Smoking	30 (44.11)	38 (57.57)	1 (Reference)	0.15
Smoking	48 (53.93)	41 (46.00)	1.58 (0.83 – 3.0)	

TABLE 6. The association between serum MMP-1 levels, age, and risk of cancer.

Variables	*r-value*[a]	*p-value*	β[b]	*p-value*	*OR*	*(95% CI)*
MMP-1	-0.092	0.22	-0.109	0.22	0.89	0.75-1.07
Age	0.162	0.005	0.022	0.005	1.02	1.006-1.038

[a]Spearman correlation between MMP-1 levels, age, and risk of cancer.
[b]Logistic regression analysis where cancer was used as a dependent variable, and MMP1 and age, were taken as independent variables.
CI = confidence interval.

between the grade and the stage of disease and tumor size with mean serum MMP-1 level. There was no association between the polymorphism of *MMP-1* (–1607) and the risk of BC and the effect of the polymorphism on serum MMP-1 levels. This observation suggested that MMP-1 levels of genetic variants in the current study were unrelated to *MMP-1* (–1607) polymorphism. It is shown that polymorphism in the promoter region of genes such as 2G allele in *MMP-1* (–1607) is associated with a variety of malignancies and cancers. The 2G allele of the *MMP-1* gene has a higher and more significant activity compared with the 1G allele by creating an active transcriptional site. This increases the risk of some cancers including oral, colorectal, kidney, and head and neck[13,15,17,18]. Even in ovarian and colorectal cancers, the presence of 2G alleles was significantly associated with a decrease in patient's longevity[14,17]. There are various reports about the association between *MMP-1* (–1607) polymorphisms and the risk of BC. Thus, the association is still unclear. In some studies, an increased risk of BC is suggested, while in others, a lack of association between the polymorphism and an increased risk of BC is observed. Kader et al[19] investigated the association between *MMP-1* polymorphism and various other metalloproteinases and BC. The age, gender, and genotype of *MMP-1* (–1607) polymorphisms relationships were investigated in the two groups of superficial and advanced cancer and no significant association was found. There was only a significant association in smoking between the two groups[19].

TABLE 7. The correlation between MMP-1 serum level and variables such as age, gender, smoking, and grade and stage of cancer.

Control Group	*r-value*	*p-value*
Age[a]	-0.009	0.93
Gender[b]	0.123	0.25
Smoking[b]	-0.017	0.87
Case Group	*r-value*	*p-value*
Age	-0.16	0.13
Gender	0.14	0.18
Smoking	0.007	0.94
Grade	-0.009	0.93
Tumor size	-0.138	0.20
Stage	-0.084	0.43

[a]Pearson correlation between quantitative traits.
[b]Spearman correlation between categorical traits.

According to a meta-analysis by Tao et al[20] on the association between the *MMP-1* polymorphism and the risk of BC, MMP-1 heterozygote showed a reduced risk of BC. An inverse association was reported between 2G alleles and the risk of BC.

Yan et al[21] stated that the *MMP-1* (–1607) polymorphism only increases the risk of BC in a recessive model, while in other conditions, no association could be found between the risk of BC and *MMP-1* polymorphism. A significant reduction in the risk of BC was observed only in 2G/1G+1G/1G genotypes by Wieczorek et al Reduction of the risk of BC in this genotypic combination was observed in smokers. There was no association between the grade and stage of the disease and genotypes[22].

Some studies delineated the association between *MMP–1* polymorphism and the susceptibility to BC.

TABLE 8. The comparison of serum MMP-1 levels in genotypes of the study groups.

Genotype	*Case Mean ± SD (Median)*	*Control Mean ± SD (Median)*	*p-value*
1G/1G	2.979 ± 2.45 (2.224)	3.148 ± 1.88 (2.413)	0.16
1G/2G	2.632 ± 1.35 (2.193)	2.704 ± 0.85 (2.615)	0.19
2G/2G	2.410 ± 1.27 (2.043)	3.128 ± 2.08 (2.538)	0.08

In the study by Srivastava et al[23] the 2G allele was observed as a high-risk allele. There was no association between the stage of disease and genotype. The 2G/2G genotype was associated with an increased risk of BC among smokers resulting in a three-fold higher risk for cancer among them. In another study, a significant association was observed between a high risk of BC incidence and 2G/2G genotype. In fact, an increase of approximately 2.7-fold was found for 2G/2G genotype in BC, but no such association was observed between the 2G/2G genotype and the grade and stage of the tumor. A three-fold risk of this genotype was observed in smokers[24]. In several studies, similar to the current one, the association between smoking and the three-fold risk of bladder cancer is reported[19,23,24].

There is no published report so far on measuring serum MMP-1 levels and its association with genetic polymorphism in BC. The serum MMP-1 levels vary according to the pathological conditions of the disease in such a way that in some cancers (BC) MMP-1 levels are lower in patients compared with healthy subjects [25,26], similar to the current study results, and in some cases (such as gastric cancer), it increases [30]. In a study by Decock et al[25] on BC, a reverse association was observed between serum level of MMP-1 and tumor size. In another similar study on BC the association between low level of serum MMP-1 and adverse prognosis in patients was also observed. Further, an inverse correlation between the serum MMP-1 levels and diagnostic factors such as tumor size, lymph node involvement, and anti-P53 antibody was observed. It was suggested that a low level of MMP-1 could be used as a negative diagnostic factor in BC[26].

An analysis of serum MMP-1 levels in patients with melanoma showed that MMP-1 levels were significantly higher in the control subjects than in the patient group. There was no correlation between the mean serum MMP-1 level and the size of tumor and metastasis[27-29].

In contrast, the level of MMP-1 in patients with gastric cancer before gastrectomy was significantly higher than that of the controls. Meanwhile, the mean serum level was closely associated with tumor size, stage, and grade of the tumor[30]. According to the results of the current study, there seems to be a reverse association between serum and tissue levels of MMP-1. Probably, the low serum level of MMP-1 in patients with cancer, compared with the healthy subjects, is due to the increase and accumulation of this enzyme in tumor tissue and its function and effect on cancerous tissues mentioned in other studies [25]. The different results obtained in different studies about the role of *MMP-1* gene polymorphism in BC susceptibility can be due to the differences in population size, genetics of individuals, and the type of method. On the other hand, the effect and interactions between the genes, the effect of the environment on the expression of *MMP-1* and its related genes, and different polymorphic loci of *MMP-1* gene can lead to different levels of cancer risk [20].

CONCLUSIONS

In the current study, there was no association between the polymorphism (*MMP-1*) and the risk of BC. Further, the current study showed that an insertion of a G-nucleotide had no effect on the level of MMP-1 in the related genotype. It is possible that other polymorphisms of *MMP-1* gene are effective in altering the expression of the enzyme among the genotypes. Certain unknown polymorphisms may also affect the expression of *MMP-1* in different genotypes. A reduction in the serum level of MMP-1 in the patient group compared with the control group may be due to the specific accumulation of this enzyme in tumor tissue and its contribution to the development of cancer. More studies in this field would help to understand more about bladder cancer biology and treatment strategies

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Conflict of Interest:
The authors declared no conflict of interest.

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