Association of Macrophage Inhibitory Factor 173- Gene Polymorphism with Biological Behavior of Prostate Cancer

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Purpose: Chronic inflammation is an important factor in the etiology of prostate cancer. Macrophage migration inhibitory factor (MIF) plays an important regulatory role in inflammatory responses. The aim of this study was to investigate the potential association between MIF-173 G/C polymorphism, and both biological behavior and incidence of prostate cancer.

Materials and Methods: Analysis of polymorphic variants for MIF was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in 128 subjects with prostate cancer and 135 controls.

Results: The frequency of MIF-173 *C allele was significantly (OR = 2.18, 95% CI = 1.32-3.61) higher in patients with prostate cancer (19.5%) than in healthy individuals (10%). Prostate cancer patients with Gleason scores ≥ 7 had higher frequency of MIF-173 *C allele than Gleason scores < 7 (86.1% vs. 27.1%, *P* = 0.003, OR = 3.18, 95% CI = 1.46-6.95). The frequency of MIF-173 *C allele was significantly different in patients with T1, T2 and ≥T3 clinical stages of prostate cancer (15.2% vs. 42.6% and 47.8%, *P* = 0.003).

Conclusion: Our data suggest that MIF-173 polymorphisms may be associated with a higher incidence of prostate cancer compared to controls. We believe that MIF-173 GC+CC genotype can be used as a predictive factor for aggressive behavior of prostate cancer including pathological stage and Gleason scores as well as metastatic potential.

Keywords: macrophage migration inhibitory factor (MIF); prostate specific antigen; polymorphism; prostate cancer

INTRODUCTION

Prostate cancer (CaP) is the most common malignancy in males aside from skin cancer and is the second leading cause of cancer mortality in the United States. The incidence of prostate cancer in Iran is close to that of Asian countries and remarkably lower than developed countries[(1)]. The aggressiveness and metastatic behavior of CaP is variable, with a spectrum that ranges from indolent cancer confined to the prostate, to cases with rapid, extra-prostatic extension and distant metastasis[(2)].

Previous studies suggested that clinical progression of CaP may be influenced by changes in expression and response to cytokine and growth factor receptors, which can be modulated by inflammatory signals[(2)]. Macrophage migration inhibitory factor (MIF) is a member of the transforming growth factor-β (TGF-β) superfamily, which is considered a pleiotropic cytokine that is a central regulator of innate immunity acts as an upstream regulator of many other inflammatory cytokines[(3)].

It is suggested that an association exists between MIF genotypes that result in increased MIF protein production and an increased risk of prostate cancer[(4-5)].

A -173 G/C substitution results in a single nucleotide polymorphism (SNP) which influences MIF gene expression[(6)]. There is accruing evidence for the relevance of this polymorphism as high-expression MIF alleles may influence biological behavior and metastasis of prostate cancer[(7-8)]. Although the exact physiologic function of MIF in tumor progression is unknown, macrophage-derived angiogenic activity may have a role[(9)]. The aim of this study was to examine the association between MIF -173 G/C polymorphism and the stage and grade of prostate cancer.

PATIENTS AND METHODS

Study population

A total of 128 subjects with prostate cancer and 135 benign prostatic hyperplasia controls were consecutively recruited from Tajrish Hospital between January 2013 and December 2016. Sample size was calculated with PASS-11 software, with 0.9 study power and OR =2.9.[(7)] All urology clinic patients with diagnosed prostate cancer who consented to participate in the study, donated 5 mL of blood. Ultrasound-guided transrectal needle biopsy of prostate (13-fold biopsy), PSA (free

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Table 1. Baseline characteristics of patients of both groups

Characteristics [a]	Cases (n = 128)	Controls (n = 135)	*P* value
Median age, year ± SD	72.6 ± 8.9	70.4 ± 8.1	0.07
Smoker, N(%)	44 (34.4)	34 (25.2)	0.1
Positive family history for cancer, N(%)	8 (6.3)	6 (4.4)	0.5
Mean PSA, ng/ml ± SD	30.1 ± 56.6	1.1 ± 0.8	< 0.001
PSA, ng/ml N(%)			
< 10	46 (35.9)	- -	
10-20	41 (32)		
> 20	41 (32)		
Gleason sum N(%)			
< 7	61 (47.7)	- -	
≥ 7	67 (52.3)		
Clinical stage N(%)			
Localized (T1-T2)	70 (54.7)	- -	
Locally advanced (T3-T4)	19 (14.8)		
Metastatic (N+ and/or M+)	39 (30.5)		

[a] Data is presented as mean ± SD or number (percent)

and total), physical and other auxiliary examinations were performed for all cases. Gleason score of surgery specimen was used for patients who underwent radical prostatectomy.

Control subjects were recruited from other patients with lower urinary tract symptoms and were frequency-matched to cases on age and smoking status. Any controls with abnormal appearance of pathology, prostate-specific antigen test > 2.5 ng/ml, abnormal digital rectal examination, other previous cancer diagnosis, history of urinary tract infection and urethral stricture disease were excluded from the study. Patients with complaint of pain in the perineum, testicles, tip of the penis, blow the wrist in pubic and bladder area, pain during urination or during or after ejaculation were also excluded. Written informed consent was provided for each participant. The research protocol was approved by the institutional review board of Shahid Beheshti University of Medical Sciences.

Genotyping

Five ml of peripheral blood was collected from the study subjects to EDTA tubes; lymphocytes were obtained from these samples and were used to isolate DNA by a salting-out procedure with minor modifications (10). Analysis of polymorphic variants for MIF was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR reaction used oligonucleotide pairs restricting the polymorphic site of the studied gene to the following sequence: MIF173: F; 5'ACTAAGAAAGACCCGAGGC-3', MIF173: R; 5'GGGGCACGTTGGTGTTTA-3'.

Briefly, PCR reaction was carried out in a total volume of 25 µL containing 1 µL of genomic DNA solution, 20 mmol/LTris–HCl (pH 8.3), 100 mmol/L KCl, 3 mmol/L MgCl2, 500 µmol/L of each dGTP, dATP, dTTP, and dCTP, 1 µl of each forward and reverse primers, and 0.1 U Taq polymerase/ml. The condition of PCR was as follows: initial denaturation at 94˚C for 3 min followed by 30 amplification cycles at 94˚C for 30 sec, 55˚C for 30 sec, and 72˚C for 60 sec, and a final extension at 72˚C for 5 min.

Amplified PCR product (3 µl) was digested in a 10-µl final reaction volume using 1 µl of 10× Reaction Buffer 2 and 4 units of Alu I restriction enzyme, at 37°C overnight. The digested products were resolved on a 3% agarose gel stained with ethidium bromide and visualized using UV transillumination.

Statistical analysis

Data were analyzed using the SPSS Software (Statistical Package for the Social Sciences, version 20.0, SPSS Inc, Chicago, IL). Chi-square test and Fisher's exact test were used to compare dichotomous variable between two groups. Independent t-test was used to comparing continuous variable between two groups. Hardy–Weinberg equilibrium was tested by Chi-Square test for both study groups. A *P*-value of < 0.05 was considered statistically significant.

Table 2. Genotype and allele frequencies of MIF polymorphisms among the cases and controls

MIF -173 G>C (%)	Cases (n = 128) [a]		Controls (n = 135) [a]		*P*-value[b]	OR (95% CI)
	Number	Percent	Number	Percent		
GG	84	65.6	110	81.5	0.01	Ref.
GC	38	29.7	23	17		2.16 (1.20-3.90)
CC	6	4.7	2	1.5		3.93 (0.77-9.96)
GC+CC	44	34.4	25	18.5	0.003	2.3 (1.31-4.07)
G allele	206	80.5	243	90	0.002	Ref.
C allele	50	19.5	27	10		2.18 (1.32-3.61)

[a] The observed frequencies among the prostate cancer and control subjects were in agreement with the Hardy-Weinberg equilibrium (X^2 = 0.394, p = 0.529 and X^2 = 0.386, p = 0.534)

b Chi-square test for either genotype distributions or allele frequencies between the cases and controls.

RESULTS

The general demographic characteristics of the case and control groups are shown in **Table 1**.

The mean ± SD age of the prostate cancer and control groups was 72.61 ±8.9 years and 70.91 ± 9.34 years respectively (P = 0.07). There was no difference in smoking history as well as first degree relative history for cancer between prostate cancer and control groups. The mean ± SD of serum total PSA levels were 30.1 ± 56.6 ng/ml in prostate cancer and 1.1±0.8 ng/ml in control subjects ($p < 0.001$). Sixty one (47.7%) prostate cancer patients had Gleason sum < 7 and 67 (52.3%) had Gleason sum ≥ 7. Pelvic CT scan was positive for lymphadenopathy in 14 (10.9%) patients and 35 (27.3%) cases had metastasis in whole body bone scan. Treatment modalities were active surveillance and watchful waiting in 9 (7%), radical prostatectomy in 48 (37.5%), radiation in 13 (10.2%), hormone therapy in 39 (30.5%), radical prostatectomy followed by hormone therapy in 12 (9.4%), radiotherapy plus hormone therapy in 7 (5.5%) and chemotherapy in 2 (1.6%) prostate cancer patients.

The allele and genotype distribution at position-173 of the MIF gene in the prostate cancer and control groups are shown in **Table 2**. No evidence of departure from Hardy-Weinberg equilibrium in in the prostate cancer and control groups was seen. The frequency of MIF-173 *C allele was significantly higher in patients with prostate cancer (19.5%) than in healthy individuals (10%).

The genotype distribution at position-173 of the MIF gene according to Gleason score and clinical stages are shown in **Table 3**. Prostate cancer patients with Gleason scores ≥ 7 had higher frequency of MIF-173 GC+CC genotype than Gleason scores < 7 (44.9% vs. 21.3%, P = 0.003, OR = 0.32, 95% CI = 0.14-0.68). The frequency of MIF-173 GC+CC genotype was significantly different in patients with T1, T2 and ≥ T3 clinical stages of prostate cancer (15.2% vs. 42.6% and 47.8%, P = 0.003). The frequency of MIF-173 GC+CC genotype in cases with regional lymph node involvement in imaging or pelvic lymph node dissection and patients with metastasis were 57.1% and 60% respectively (p=0.075 and < 0.001, OR=0.35 CI = 0.11-1.07 and OR=0.22 CI = .09-0.49, respectively).

DISCUSSION

In the present study we investigated the association between the MIF -173 G/C polymorphism and both incidence and behavior of prostatic carcinoma. MIF is a multifunctional cytokine which has a regulatory role in inflammatory response[11] and stimulates secretion of other proinflamatory mediators such as TNF α and IL1[12]. Because the correlation between chronic inflammation and cancer has been established[13] and also angiogenic effects of MIF[14], the association of MIF and cancers was studied in some investigations. The correlation between MIF and prostate [2,7], gastric[15], breast [16] and bladder[17] cancer and acute lymphoblastic leukemia[4] has been shown in some studies. It seems that MIF promotes tumor survival by inducing an angiogenic response, but MIF is not directly angiogenic.

Prostatic adenocarcinoma is the most commonly diagnosed non-cutaneous malignant tumor. Some studies have reported higher expression of MIF gene in prostate cancer tissue than in normal prostate tissue[18].

Meyer-Siegler and colleagues found enhanced MIF mRNA levels in metastatic adenocarcinoma of prostate in comparison with normal prostatic tissues[19]. They postulated that this cytokine plays a role in the development of metastasis and it may represent a prognostic factor for prostate cancer. In another study they showed higher MIF expression in metastatic adenocarcinoma than in the normal prostate, BPH or focal prostate adenocarcinoma[20]. This increased serum MIF concentrations in patients with prostatic adenocarcinoma was irrespective of treatment modality indicating that continuing MIF secretion by the prostate cancer epithelial cells may not be regulated hormonally. The association between MIF expression and tumor grading and prognosis of prostate cancer was identified in another study [21].

The MIF -173 G/C polymorphism was identified and higher serum MIF levels were found in subjects with MIF -173 *C compared to the MIF -173 GG genotype [22-23]. Meyer-Siegler at al.[2] evaluated the correlation between -173C and -794 7-CATT polymorphism and prostate cancer. They reported that MIF gene polymorphism was associated with incidence and also grading of prostate cancer. Individuals with -173*C genotype had a higher grade (Gleason score ≥7) prostate cancer when compared to those that had the G/G genotype [OR=9.69; 95%CI: 2.20-42.66].

Table 3. Frequency distributions among Gleason Scores and clinical stages of prostate cancer between the genotypes of the MIF polymorphisms

	GG (n = 84)		GC+CC (n = 44)		*P* value	OR (95% CI)
	Number	Percent	Number	Percent		
PSA (ng/ml)						
< 10	38	45.2	8	18.2	<0.001	Ref.
10-20	29	34.5	12	27.3		0.51 (0.18-1.41)
> 20	17	5.9	24	9.1		0.15(0.06-0.40)
Gleason sum						
< 7	48	57.1	13	29.5	0.003	Ref.
≥ 7	36	42.9	31	70.5		0.32 (0.14-0.68)
Clinical stage						
Localized (T1-T2)	66	78.5	4	9.1	0.003	Ref.
Locally advanced (T3-T4)	7	8.4	12	27.2	<0.001	1.16(0.44-3.06)
Metastatic (N+ and/or M+)	11	13.1	28	63.7		0.22(0.09-0.49)

Ding and colleagues[7] evaluated the association of MIF -173 polymorphism with incidence and Gleason score, clinical stage and PSA value of prostate cancer. They showed that C allele carriers are at higher risk for prostate cancer [OR=3.27; 95%CI: 2.13-4.47]. This suggests that MIF -173 polymorphism may play a role in the etiology of prostate cancer. They considered MIF may contribute in tumorogenesis through its ability to antagonize P53 which was previously shown in some studies[24-26]. Moreover MIF over-expression due to MIF polymorphism may promote chronic inflammatory response and the resultant cancer[13].

Arisawa et al.[27] evaluated 229 patients with gastric cancer and 428 subjects with no evidence of gastric malignancies on the upper gastro-duodenal endoscopy and reported an association between the -173C MIF allele and gastric cancer in patients older than 60 years [OR=1.71; 95%CI: 1.03-2.84]. Ziino et al.[28] failed to find a significant association between the MIF −173G/C polymorphism and prednisone poor response in childhood ALL. Xue et al[4] compared 346 acute lymphoblastic leukemia (ALL) cases and 516 cancer-free controls and showed that the variant genotype GC and the combined genotypes GC/CC were associated with a significantly higher risk of childhood ALL [OR=1.39, 95% CI:1.01-1.93 for GC and adjusted OR=1.38, 95% CI:1.01-1.89 for GC/CC]. Vera and Meyer-Siegler[3] in a meta-analysis suggested that the -173C MIF promoter polymorphism is associated with an increase in the risk of solid tumor cancer, particularly for prostate cancer but not for "non-solid" tumors (leukemia). In contrast, Yuan et al[17] compared 325 patients with bladder cancer with 345 cancer-free controls and found that MIF-173C alleles associates with decreased risk of bladder cancer [OR = 0.57, 95% CI, 0.41-0.79].

Sadjadi and his colleagues showed that based on geographical distribution, the prevalence of prostate cancer in Iran is lower than in Western Countries[1]. However, after one decade from that research, Pakzad et al. found that there was a significant increase in the incidence of PCa, with annual percentage increase of 17.3%[29]. These finding have led us to explore further research on cellular genetics of prostate cancer in our country.

We found correlation between MIF -173*C genotype and higher Gleason scores and PSA values and advanced clinical stages which were similar to Meyer-Siegler[2] and Ding's study[7]. Our results suggest that MIF -173C polymorphism may have predictive value for behavior of prostate cancer. Low number of patients in both prostate cancer and control groups was a major limitation of our study.

CONCLUSIONS

In this study we showed the association between MIF -173C polymorphism and incidence and behavioral characteristics of prostate cancer such as Gleason score and clinical stage. We believe that MIF -173C polymorphism correlates with higher incidence of prostate cancer and also can be used as a predictive marker for aggressive behavior of prostate cancer independent of Gleason score and clinical stage. However our findings support the need for larger studies underlining the predictive value of MIF -173C polymorphism in prognosis of prostate cancer.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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