

# Macrophage Migration Inhibitory Factor (MIF) Gene Polymorphism of Urothelial Carcinoma Patients in Iraq

Ishraq Abdul Ameer Salih

Assistant prof., Department of Biology, Science For Women, University of Babylon

## Abstract

Bladder cancer is the 2nd most frequent malignancy of the genitourinary tract and the fourth most common cancer in men. This study was designed to shed light on the association between polymorphism of macrophage migration inhibitory factor (MIF) gene at -173 locus as risk factor of bladder cancer in Iraqi population by PCR-RFLP. In a hospital-based case-control study of 70 patients with bladder cancer and 30 cancer-free controls frequency-matched by age, sex, smoking status, and alcohol use, we genotyped the MIF polymorphism and analyzed immunohistochemical stained operational bladder cancer tissue sections for MIF. We found that individuals with GC/GG genotype had a significantly decreased risk of bladder cancer (OR = 1.55, 95% CI, 0.498 to 8.85) than those with CC genotype. The frequencies of the C allele on the MIF gene were significantly increased in Urinary bladder carcinoma patients. MIF -173G>C polymorphism may play a role in the etiology of bladder cancer in Iraqi.

**Keywords :** MIF gene, RFLP, Urinary bladder carcinoma.

## Introduction

Urothelial cell carcinoma occurs in the cells that line the inside of bladder. Bladder cancer is the ninth most frequent cancer, and it is estimated that there were 340,000 newly diagnosed bladder cancer cases and 130,000 related deaths world wide in <sup>1</sup>. MIF enhancement of macrophage transcription, activation and viability, coupled with its inhibitory effects on anti-tumor cell cytotoxic lymphocytes, suggests that MIF overexpression in developing malignancies may act in concert to facilitate increased tumor growth which present an important link between inflammation and cancer due to its pro-inflammatory role. Its molecular mechanisms involve, among others, the inhibition of p53 which promote tumor cell proliferation, cell survival and tumor-associated neoangiogenesis <sup>(2, 3)</sup>. Macrophage migration inhibitory factor (MIF) functions as a pleiotropic protein, participating in inflammatory and immune responses. MIF was originally discovered as a lymphokine involved in delayed hypersensitivity and various macrophage functions, including production of proinflammatory cytokines, glucocorticoid-induced immunomodulator, and natural killer cell inhibitory factor (NKIF), regulation of toll-like receptor expression, adherence and phagocytosis of macrophages, as well as

induction of metalloproteinase. <sup>4</sup>. The imbalance in the regulation of inflammation that occurs in many cancers can induce cellular damage. This stimulates interaction between immune cells and the damaged cells, which then proliferate, invade, and subsequently develop into tumors <sup>5</sup>. As a functional promoter, the MIF-173 situate in the 50 flanking region of MIF gene, which is strongly associated with protein production. In 2007, Meyer-Siegler et al. <sup>6</sup> reported that the MIF allele defined by -173C SNP was associated independently with prostate cancer and independently with elevate levels of circulating MIF. The human MIF gene situated on 22q11.2 having a single nucleotide polymorphism (SNP; G to C transition) in the 50-flanking region at position -173, which has been associated with susceptibility to adult inflammation <sup>7</sup>. In 2005, Ziino et al. <sup>8</sup> revealed that the MIF-173 G[C polymorphism did not contribute to prednisone poor response in vivo in childhood acute lymphoblastic leukemia. Approximately 20% of all cancers arise in association with infection and chronic inflammation and even those cancers that do not develop as a consequence of chronic inflammation, exhibit extensive inflammatory infiltrates with high levels of cytokine expression in the tumor microenvironment.

## Materials and Method

### Sampling and data collection

This a case-control study consisted of 70 patients with RA and 30 healthy persons (before surgery for UBC group). All subjects signed an informed consent, and clinical data of patients were collected from patient files and questionnaires. Our study was approved by the Research Ethics of the Iraqi Ministry of Health. About 2 ml of whole blood was collected from all subjects

### DNA extraction and purification

Genomic DNA was extracted from whole blood collected in EDTA-tubes from all subjects (patients and control individuals) using Genomic DNA Extraction Blood DNA Mini Kit (FAVORGENE). The concentration (ng/ml) and purity (260/280 nm) of the DNA extracts were measured at 260 nm and 280 nm with a NanoDrop spectrophotometer (OPTIZEN POP – Korea).

### Genotyping

The candidate SNP *MIF*<sub>-173</sub> was investigated using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The amplification reaction was done with 3 µl of 200ng/µl of genomic DNA, 12.5 µl of 1X Master Mix (Promega), 1.5 µl of 10 pmol of each specific primer pair (forward and reverse primer), and completed the volume of 20 µl PCR mixture with DNase free water. The source of the primer was Bioneer (South Korea). Primer sequences of *MIF*<sub>-173</sub> were forward (5'- 5-ACTAAGAAAGACCCGAGGC-/3) and reverse (5'-GGGGCACGTTGGTGTTTA ')<sup>8</sup>.

The technique for PCR included a pre-denaturing temperature at 95°C for 10min, followed by 30 cycles at 95°C for 45 s; 62°C for 45 s; and 72°C for 30 min with a final extension at 72°C for 10 min. Ten µl of PCR products (366 bp in length) was digested according to Promega company protocol, which the digestion reaction mixture (36 µl) composed of 0.5 µl *Alu I*, 2 µl of buffer B, 0.2 µl BSA buffer, 7 µl of 1X Muti core buffer and 16.3 µl of DH<sub>2</sub>O; the reaction was incubated at 37°C for 16h. Subsequently, The product was separated on a 1.5% agarose gel for 45min and power 70 volt and 20mA. Finally, The gel was viewed by RedSafe™ Nucleic Acid Staining (iNtRON) under ultraviolet light . DNA Marker;G/G:Homozygous for absence of *AluI* restriction site (268bp 98bp); C/C: Homozygous for *AluI* restriction site (206bp, 98bp,62Heterozygous for *AluI* restriction site (268bp,206bp,98bp,62bp).

### Statistical analysis

Two-sided x2 test was used to evaluate the frequency distributions of select demographic variables, smoking status, alcohol use, and alleles and genotypes of MIF polymorphism between the test subjects and controls. The associations between MIF genotypes and the risk of bladder cancer were estimated by computing the crude and adjusted odds ratios (ORs) and their 95% confidence intervals (95% CIs) from logistic regression analyses.

## Results and Discussion

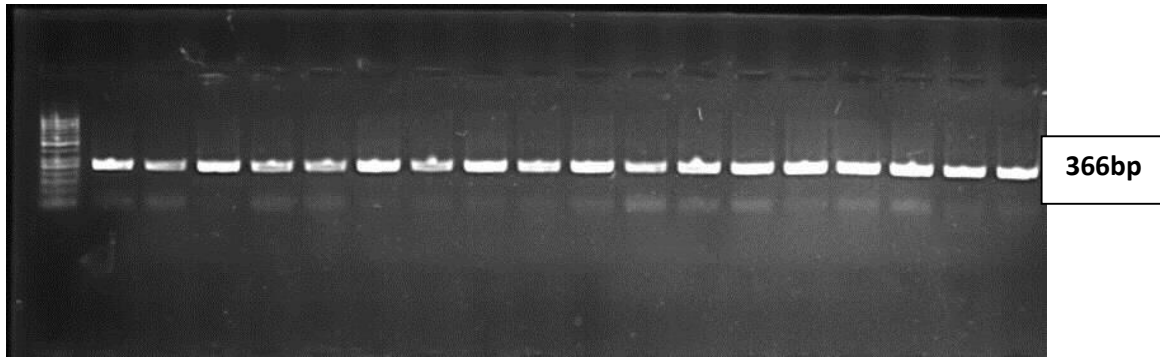
The frequency of genotypes distribution and alleles of the groups are presented in table (3-18). The GG, GC, and CC genotype frequencies were 41.4%, 10% and 44.2%, respectively among the UBC cases and 67.5, 15, and 17.5%, respectively, respectively among healthy cases , as shown in table (3)

**Table (1) Distribution of allele frequency and genotype of *MIF* in case-control study.**

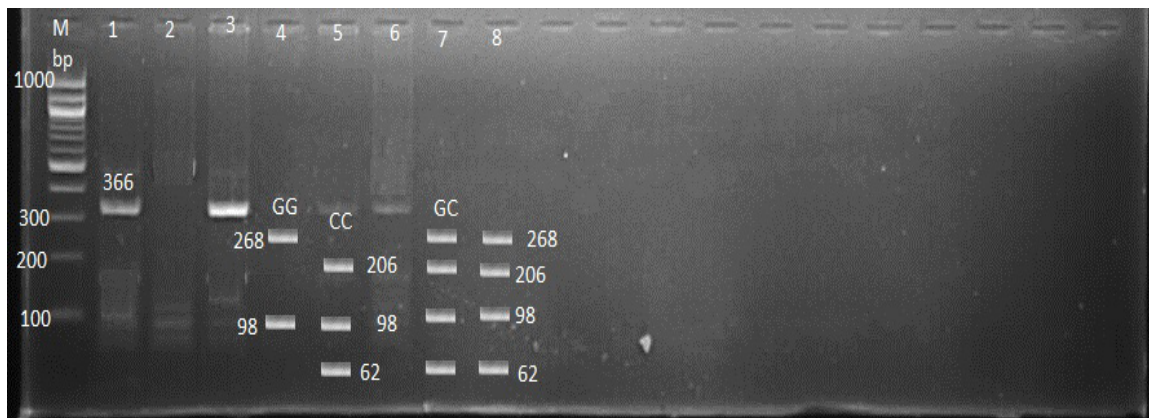
Genotype	Patients N(% 70(100%))	ControlN% 40(100%)		TEST X2	Odd ratio	CI 95%
GG	29(41.4%)	27(67.5%)		0		
GC	10(14.4%)	6(15%)		0.001*	1,55	0.498 to 8.85
CC	31(44.2 %)	7 (17.5%)			4,12	1.55 to 10.91
Allele Frequency						
G	68(49%)	54(67%)	1.8		2.411	1.3530 to 4.299
C	72(51%)	26(33%)				

OR: Odd Ratio.

CI: Confidence Interval.



**Figure (1)** Electrophoresis pattern of (MIF) Amplification using PCR technique 2% agarose, 75V, 20mA, for 60 min, line 1 (50 bp) DNA marker, line 1-8 MIF genotype for patients, lane 9-12 MIF genotype for control.



**Figure (2)** Gel electrophoresis of *MIF*<sub>-173</sub> polymorphism (RFLP by *AluI*); M: DNA Marker (100); 4, G/G: Homozygous for absence of *AluI* restriction site (268bp, 98bp); 5 C/C: Homozygous for *AluI* restriction site (206bp, 98bp, 62bp); 6, 7, 8 (268bp, 206bp, 98bp, 62bp).

The results nearly agreed with the results reported by <sup>11</sup> who found that individuals with GC/CC genotype had a significantly decreased risk of bladder cancer than those with GG genotype and the CC genotype frequency of *MIF* was lower among the cases. The GG, GC, and CC genotype frequencies were 63.4, 30.5, and 6.1%, respectively, among the cases and 50.7, 43.2, and 6.1%, respectively, among the controls.

The human *MIF* gene is located on chromosome 22q11.2 which contains 3 exons and 2 introns. Gene mapping has shown that the *MIF* gene displays polymorphism, including microsatellite polymorphism and single nucleotide polymorphism, at 4 sites (11,12). G/C polymorphism is located at -173, T/G polymorphism is located at +254, C/G polymorphism.

Although, how the *MIF*-173 G/C polymorphisms affect the inflammatory factor activity remains to be investigated, some studies suggested that the polymorphisms may affect the protein functions. Arikan

et al. <sup>14</sup> revealed that *MIF*-173C allele frequency was significantly higher in biliary atresia patients than both the chronic liver disease patients and healthy control groups. Makhija et al. <sup>15</sup> reported that the *MIF*-173C alleles was over expressed in acute pancreatitis patients. Their data all suggested that the polymorphisms might affect on *MIF* protein expression and activity.

### Conclusion

We found that individuals with GC/GG genotype had a significantly decreased risk of bladder cancer (OR = 1.55, 95% CI, 0.498 to 8.85) than those with CC genotype. The frequencies of the C allele on the *MIF* gene were significantly increased in Urinary bladder carcinoma patients. *MIF* -173G>C polymorphism may play a role in the etiology of bladder cancer in Iraqi.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Biology, Science For Women, University of Babylon and all experiments were carried out in accordance with approved guidelines.

### References

1. Nuno M , López-Knowles L , F Real. Molecular Biology of Bladder Cancer. *Clin Transl Oncol* . 2007; 9:5-12.
2. Maynard C , Harrington K, Janowski J, Oliver M , C Zindl. Regulatory T cells expressing Interleukin 10 Develop from Foxp3+ and Foxp3- Precursor Cells in the Absence of Interleukin 10. *Nat. Immunol.* 2007;8: 931-941.
3. Souza M , Curioni O , Kanda J , M De Carvalho. Serum and Salivary Macrophage Migration Inhibitory Factor in patients with Oral Squamous Cell Carcinoma. *Oncology Letters* . 2014;8 ( ): 2267-2275.
4. Liu Y , J Lin. Recent Advances of Cluster of Differentiation 74 in Cancer. *World J Immunol* 2014 November . 2014; 4(3): 174-184.
5. Morris K , Nofchissey R , Pinchuk I , E Beswick . Chronic Macrophage Migration Inhibitory Factor Exposure Induces Mesenchymal Epithelial Transition and Promotes Gastric and Colon Cancers. *Plos One* . 2014.
6. Sjudah G , Lauss M , Lovgren K , Chebil G, Gudjonsson S . A Molecular Taxonomy for Urothelial Carcinoma. *Clin Cancer Res* . 2015; 18(12):3376-3386.
7. Chen Z , Zhou W , Dai M , W Jin . Association between the Interaction polymorphisms of interleukin-10 and Smoking on patients with Bladder Cancer Risk from a Case Control Study. *Zhonghua Liuxingbingxue Zazhi* . 2013;34(2):183-186.
8. Ziino O, D'Urbano LE, De Benedetti F, Conter V, Barisone E, De Rossi G, Basso G, Arico M . The MIF-173G/C polymorphism does not contribute to prednisone poor response in vivo in childhood acute lymphoblastic leukemia. *Leukemia* 2005;19: 2346–2347
9. Hizawa N , Yamaguchi E , Takahashi D , Nishihira J , M Nishimura . Functional Polymorphisms in the Promoter Region of Macrophage Migration Inhibitory Factor and Atopy. *Am J Respir Crit Care Med* . 2004;169: 1014–1018.
10. Grieb G ,Merk M , Bernhagen J , R Bucala . Macrophage Migration Inhibitory Factor (MIF): a promising Biomarker. *Drug News Perspect.*; 2010;23(4): 257–264.
11. Yuan T, Tang C, Chen M, Deng C, P Chen. Influence of The Human MIF Promoter Polymorphism on Hepatocellular Carcinoma prognosis. *Genetic and Molecular Research*. 2013;12 (4): 6629- 6635.
12. Das R , Subrahmanyam L , Ivana V , Duin D , Levy R , Piecychna M. Functional Polymorphisms in the Gene Encoding Macrophage Migration Inhibitory Factor Are Associated With Gram-Negative Bacteremia in Older Adults. *The Journal of Infectious Diseases* . 2014 ;209:764–768.
13. Ishraq Abdul Ameer Salih<sup>1</sup> , Rabab Omran “ Vascular Endothelial Growth Factor / Vascular Permeability Factor and VEGF Gene polymorphisms is Detectable Rheumatoid Arthritis patients “ *Indian Journal of Public Health Research & Development*, 2018;1(10).
14. Arian C, Berdeli A, Ozgenç F, Tumgor G, Yagci RV, Aydogdu S . Positive association of macrophage migration inhibitory 3114 Mol Biol Rep (2012) 39:3109–3115 123 factor gene-173G/C polymorphism with biliary atresia. *J Pediatr Gastroenterol Nutr* .2006;42:77–82
15. Makhija R, Kingsnorth A, Demaine A . Gene polymorphisms of the macrophage migration inhibitory factor and acute pancreatitis. *Jop* . 2007;8:289–295