International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Polymorphism in the Promoter Region of *MIF* and Risk of Bladder Cancer in Iraqi Patients

Rawaa AlChalabi[a]* , Shehlaa Mahdi[b], Ayad Fadhil[c] and Hayder Jawad[d]

[a]*Al-Nahrain University /College of Applied Biotechnology, Baghdad,Iraq*

[b]*Al-Nahrain University /College of Applied Biotechnology, Baghdad,Iraq*

[a]*Email: rawaaalchalabi_1984@yahoo.com*

[b]*Email:shehlaam2006@yahoo.com*

Abstract

This study was aimed to investigate gene polymorphism of macrophage migration inhibitory factor (MIF) at -173 locus as risk factor of bladder cancer in Iraqi population by PCR-RFLP. Accordingly 135 Iraqi Arab subjects were distributed as 73 patients with urinary bladder carcinoma (UBC), 32 patients with urinary bladder disorders and 30 healthy controls. The frequency distributions of genotypes and alleles of the groups showed the GG and G allele were (61.9 and 78.77) % among the UBC cases with positive association with no significant relative risk (RR) value1. 41and etiological factor (EF) value of 0.1 8and (56.3 and 75)% among the UBD cases while represent (53.3and 70)% respectively among healthy cases and no significant variation was recorded between UBC and control**.**

Keywords: *MIF* Polymorphism; Bladder cancer.

1. Introduction

Bladder cancer is abnormal cells multiply without control in the bladder arising from the epithelial lining (the urothelium) of the urinary bladder has the highest recurrence rate of any malignancy.

---

* Corresponding author.

Rarely the bladder is involved by non-epithelial cancers, such as lymphoma or sarcoma. Bladder cancer generates the highest medical cost per patient and it is the fifth most expensive cancer in terms of total medical care expenditures. Approximately 95% of malignant bladder tumors are urothelial cell carcinomas (UCC) which can be classified as papillary (most common type, tend to grow slowly towards the lumen), solid (less frequent, infiltrate the bladder wall and are more aggressive) or carcinoma In Situ (CIS, a very aggressive kind of cancer that involves only the inner lining of the bladder [1,2,3,4].

Macrophage migration inhibitory factor (MIF) is pro-inflammatory cytokine shown to promote tumorigenesis discovered in 1966. The *MIF* gene coded 12.5 kDa polypeptide and the protein consists of 115 amino acids which lies on chromosome 22q11.2 and regulation of the gene is by the two polymorphic sites in the promoter region .The first site consists of the CATT repeat at -794 which repeats 5-8 times and the second site is a single nucleotide polymorphism at -173(G/C). This site is associated with enhanced promoter activity in some cancer cell lines and the activity is proportional increased MIF levels in serum [5,6,7,8].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 [9,10]. MIF binds to extracellular domain of CD74with high affinity and initiates a signaling cascade. CD74 forms a complex with CD44which is essential for the MIF-induced signaling cascade. Rare (SNPs) in the CD74 gene have been reported, but SNPs in molecules that interact with CD74, such as MIF, CD44and MHC class are more frequent and are associated with the development of cancer. 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 [11,12].

A DNA polymorphism is a difference in the nucleotide sequence between individuals of the same species. These differences can be single base pair changes, deletions, insertions, or even changes in the number of copies of a given DNA sequence. SNPs (single nucleotide polymorphisms) are the most common type of DNA polymorphism in humans. An example of an SNP would be if a cytosine (C) nucleotide is present at a particular locus in one person's DNA but a thymine (T) nucleotide occurs at the same locus in another person's DNA. A polymorphic variant of a gene may lead to the abnormal expression or to the production of an abnormal form of the gene; this may cause or be associated with disease [13,14].

Cancer results from a series of genetic alterations leading to a progressive disorder of the normal mechanisms controlling growth, differentiation, cell death, or genomic instability. The response of the cell to genetic injury and its ability to maintain genomic stability by means of a variety of DNA repair mechanisms are essential in preventing tumor initiation and progression. Polymorphisms of several DNA repair-related genes and some cytokine genes have been found to be associated with the risk of developing different tumor types. Polymorphic alleles of DNA genes would predispose carriers to a higher risk of developing cancer but would not necessarily cause cancer. Therefore, possible gene–gene interaction and gene–risk factor interaction may play an important role in modifying the cancer risk associated with particular gene polymorphism in different study populations

[15,16,17]. The nucleotide sequences of DNAs in humans are not identical in different individuals. Nucleotide substitutions have been estimated to occur every few hundred base pairs in the human genome. Nucleotide sequence polymorphism has been detected as restriction fragment length polymorphism (RFLP). Although RFLPs are very useful for distinguishing two alleles at chromosomal loci, they can be detected only when DNA polymorphisms are present in the recognition sequences for the corresponding restriction endonucleases or when deletion or insertion of a short sequence is present in the region detected by a particular probe. In recent years, several functional polymorphisms, particularly, SNPs have been identified in cytokines and their receptor genes that regulate levels of cytokine expression. These have been implicated as immune prognostic markers in diseases, including differential response to therapy and as biomarkers of graft outcome following organ and stem cell transplantation. Population distribution of cytokine gene polymorphisms (CGPs) reveals significant variations in allele frequencies in different ethnic groups and this might explain, to some extent, the observed differences in SNP associations with various diseases and immune-pathologies [18,19,20,21]

2. Materials and Methods

2.1 Patients

A total of 73 subjects diagnosed with bladder cancer, 32 subjects had urinary bladder disorder and 30healthy controls were consecutively recruited from The AlYarmook Teaching Hospital and AlJabchi privet Hospital between March 2014 and November 2014. All cases were urology clinic patients with histological confirmed transitional cell carcinoma, were consent to participate in the study, and donated 5 mL of blood. The controls were genetically unrelated cancer-free individuals living in the same residential areas and frequency-matched to the cases on age, sex, smoking status, and alcohol use.

2.2 Genotyping

Genomic DNA was extracted from peripheral blood by Using relia –prep kit (Promega/USA) .The DNA pellet was resuspended in TE (Tris-EDTA) buffer (Promega /USA) then purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm. The MIF-173G/C polymorphism was determined by using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method. The primers designed in this study were as follows: ′5ACTAAGAAAGACCCGAGGC′3 (forward) and′5 GGGGCACGTTGGTGTTTA′3 (reverse). Amplification was carried out in 0.2 mL thin-wall PCR strip tubes (Eppendorff/Germany) containing 12.5µl of master mix , 8.5 µl ddH2O, 2 µl template DNA and 1 µl of each forward and reverse primers(Alpha DNA, Canada).The cycling conditions comprised a hot start at 95°C for5 min, followed by 35 amplification cycles at 95°C for 1min , 60°C for 1 min, and 72°C for 1mins, and a final extension at 72°C for 5 min. The PCR product was a 366 bp fragment that contained both the polymorphic and a non-polymorphic AluI site. Amplified PCR product (20µL) was digested in a 25 µl final reaction volume using 5µl of reaction buffer and 10 units of AluI restriction enzyme at 37°C for 3 hours. The gel was then stained with ethidium bromide. The digestion fragments for the polymorphisms were separated by 3% agarose gel. Since G/G gene type cannot be cut off by AluI restriction enzyme at -173, we got two fragments as 268- and 98-bp; C/C gene type can be digested by AluI at -173, so we got 62-, 206- and 98-bp three fragments; and it

turns out to be four fragments as 62-, 98-, 206- and 268-bp after digestion of G/C type by AluI.

2.3 Determination of Serum MIF

Serum level of MIF measured by using ELISA kit (R&D, USA), based upon coating wells of a high protein binding ELISA plate with monoclonal antibody specific for human MIF. Standard and sample were added to appropriate well followed by covering the plate with the adhesive strip and incubates for 2 hours at room temperature then washed four times with wash buffer. 200µl of cytokine conjugate was added to each well and covered with a new adhesive strip, incubated for 2 hours at room temperature on the shaker then washed four times . 200 µl of substrate solution was added to each well and incubated for 30 min at room temperature in dark. Finally,50 µl of stop solution was added to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing. The optical density of each well was measured within 30 minutes, using a microplate reader set to 450 nm.

3. Results and Discussion

MIF Gene Polymorphism at -173

Macrophage migration inhibitory factor (MIF) is an inflammatory factors may promote carcinogenesis which derived from T-cell, known as a member of the transforming growth factor-β (TGF-β) superfamily, plays an important role in the pro- and anti-inflammatory response to infection and in the etiology of bladder cancer[22].MIF-173 locus polymorphism might contribute to genetic susceptibility to bladder cancer. In a hospital-based case–control study of 73 patients with bladder cancer and 62 cancer-free controls then genotyped the *MIF* polymorphism. Genomic DNA were extracted from peripheral blood of all 135 subjects then amplified DNA by PCR with using specific primer for -173 locus that gave the PCR product (366) bp as shown as in Figure (1).The frequency distributions of genotypes and alleles of the groups are presented in Table(1) . The GG, GC, and CC genotype frequencies were 61.9%, 31.6% and 5.5%, respectively among the UBC cases and 56.3, 37.5, and 6.2%, respectively, among the UBD cases while represent 53.3 %,33.3% and 13.4% respectively among healthy cases.

As a protective factor, the CC genotype frequency of MIF was lower among the cases. Results nearly agreed with results reported by Yuan *and his colleagues*,(2012) who found that individuals with GC/CC genotype had a significantly decreased risk of bladder cancer than those with GG genotype. *MIF* polymorphism, 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., thus MIF -173G/C polymorphism may play a role in the etiology of bladder cancer in southern Chinese population .Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that plays a significant role in pathogenesis and autoimmune diseases and functional polymorphisms in human MIF that separate the population into those with high and those with low expression of MIF.

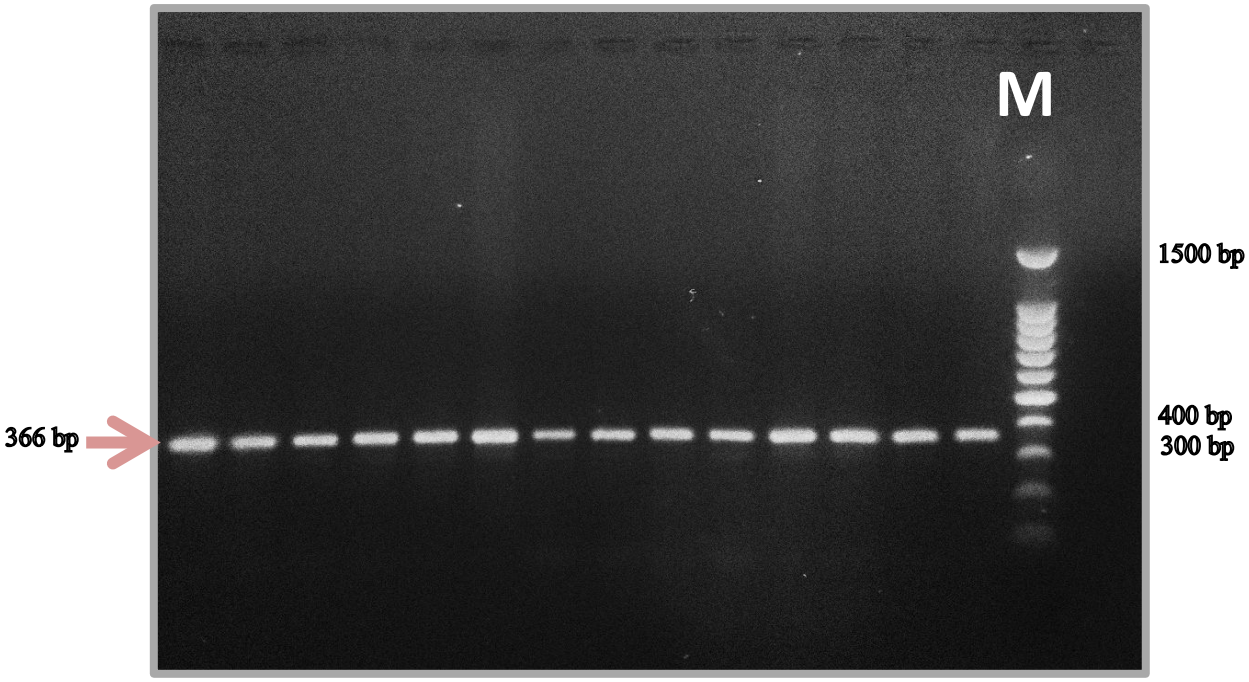

Figure1: Gel electrophoresis for amplification of human *MIF* gene.

Electrophoresis was performed on 1.2 % agarose gel and run with a 70 volt current for 2 hrs. Lane M is a (100bp) ladder

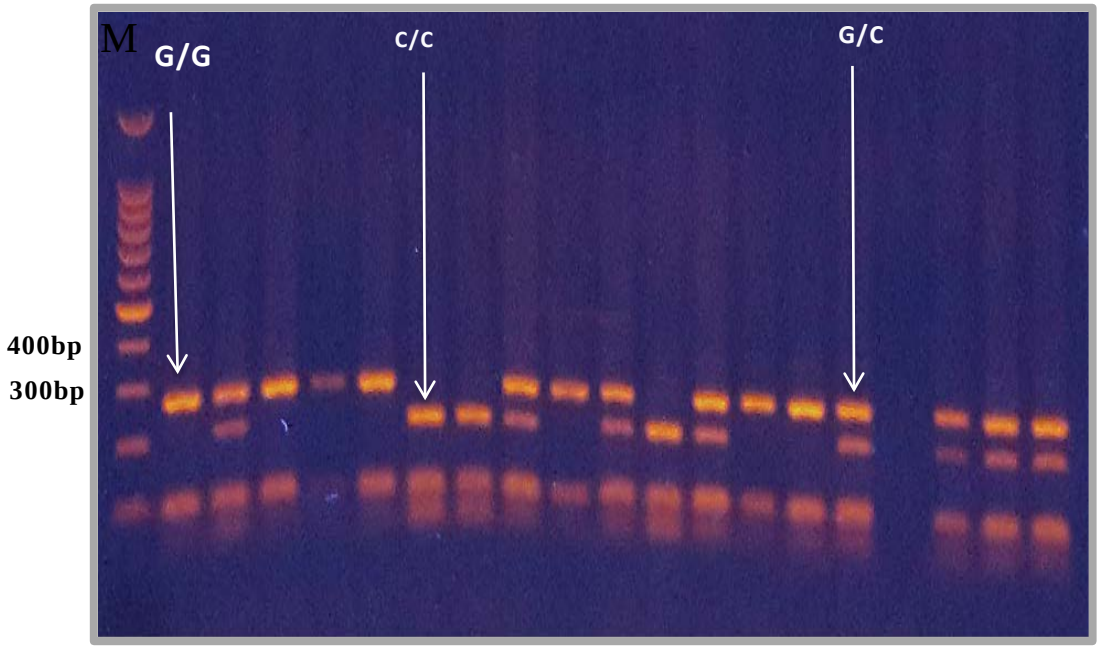

Figure 2: Gel Electrophoresis of *MIF* -173 polymorphism (RFLP by Alu1);M: DNA Marker; G/G: Homozygous for absence of Alu1 restriction site (268bp,98bp); C/C: Homozygous for Alu1 restriction site (206bp,98bp,62bp);G/C:Heterozygous for Alu1 restriction site (268bp,206bp,98bp,62bp).

Table 1: Genotypes distribution among UBC, UBD and healthy

Genotype	Groups						Total	
	UBC		UBD		Healthy		No.	%
	No.	%	No.	%	No.	%		
GG	45	61.6	18	56.3	16	53.3	79	58.5
GC	24	32.9	12	37.5	10	33.3	46	34.1
CC	4	5.5	2	6.2	4	13.4	10	7.4
Total	73	54.1	32	23.7	30	22.2	135	100

The human *MIF* gene locates on chromosome 22q11.2 and 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. G/C polymorphism locates at -173, T/G polymorphism locates at +254, C/G polymorphism locates at +656, and a CATT repetitive sequence locates at -794. −173G/C polymorphism may be associated with cancer risk[23,24,25].The genotypes and allele frequencies of *MIF* polymorphism and its association with risk of bladder cancer are summarized in table (2).

Table 2: Observed numbers alleles frequencies(Hardy-Weinberg equilibrium) of the *MIF* at -173 SNP in UBC,UBD and Control

Group		MIF-173 Genotype or Allele					H-W (P≤)
		GG	GC	CC	G	C	
UBC	Observed	No. 46	23	4	115	31	N.S
		% 61.9	31.6	5.5	78.77	21.23	
	Expected	No. 45.29	24.42	3.29	Not Estimated		
		% 62	33.45	4.5			
UBD	Observed	No. 18	12	2	48	16	N.S
		% 56.25	37.50	6.25	75	25	
	Expected	No. 18	12	2	Not Estimated		
		% 56.25	37.50	37.50			
Control	Observed	No. 16	10	4	42	18	N.S
		% 53.33	33.33	13.34	70	30	
	Expected	No. 14.7	12.6	2.7	Not Estimated		
		% 49	42	9			

N.S : Not Significant

Nishihira and his colleagues, (2003) reported that MIF has the potential to promote tumor growth and tumor-associated angiogenesis in mice and mice bladder cancer experiment, while Arikan *and his colleagues*,(2006) revealed that *MIF*-173C allele frequency was significantly higher in biliary atresia patients than both the chronic liver disease patients and healthy control groups and Makhija *and his colleagues* ,(2007) 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 thus the variation in MIF may play a role in etiology of bladder cancer. 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. The frequency of GG genotype was increased as well as G allele which had positive association with RR value1.32 and EF value 0.13 while GC and CC genotypes were decreased as well as C allele , both of them had negative association with RR value 0.78,0.69 respectively .such two negative association scored PF value as shown in table(3).

Table 3: Statistical evolution of association between *MIF* $_{-173}$ genotypes or alleles and urinary bladder carcinoma

MIF_{-173} Genotype or Allele	Statistical Evolution			
	Relative Risk	Etiological Or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
G/G	1.41	0.18	0.510	0.60-3.28
G/C	0.98	0.07	0.569	0.40-2.42
C/C	0.38	0.08	0.170	0.09-1.59
G	1.59	0.29	0.123	0.81-3.12
C	0.63	0.11	0.123	0.32-1.24

Single nucleotide polymorphisms (SNP) in inflammation genes have been shown to alter their expression and functions. A G/C SNP in the promoter region (174) of IL-6 was shown to affect transcription and alter plasma IL-6 levels. The A-allele of an IL-8 SNP in the promoter region (T-251A) has been associated with increased IL-8 production by lipopolysaccharide-stimulated whole blood. A G-to-A transition in the promoter region (308) of the TNF- gene results in higher expression of TNF.

Results showed that MIF level in GG was (62.69) pg/ml significantly higher in UBC. A significant decrease was recorded in UBD and healthy (37.98 and 16. 61 pg/ml) respectively. In GC genotype MIF level was (52.51) pg/ml followed by (38.16) pg/ml in UBD and (22.38) pg/ml in healthy with significant differences. A significant increase was recorded in CC genotype in UBC and UBD (53.54 and 54.60) pg/ml respectively in comparison in healthy (16. 57) pg/ml with significant differences ($p \leq 0.01$).

Table (4): Association between different genotypes and MIF level in UBC , UBD and healthy.

Genotype	Group (Mean level of MIF Mean± SE) pg/ml			Significant between groups	P-value
	UBC	UBD	Healthy		
GG	62.69 ± 5.52	37.98 ± 2.46	16.61 ± 0.79	13.685 **	0.0027
GC	52.51 ± 3.57	38.16 ± 4.51	22.38 ± 3.21	18.702 **	0.0001
CC	53.54 ± 6.54	54.60 ± 16.50	16.57 ± 1.78	18.963 **	0.0001
LSD -value	15.355 NS	19.050 NS	11.163 NS	----	

Different letters means significant differences between mean P≤ 0.01

Meyer-Siegler et al .,(2007) reported the concentration of MIF is higher in bladder cancer tissue than in normal bladder tissue especially in muscle invasive bladder cancer tissue and in functional promoter, the *MIF*-173 situate in the 50 flanking region of *MIF* gene, which is strongly associated with protein production. Also, *MIF* allele defined by -173C SNP was associated independently with prostate cancer and independently with elevate levels of circulating MIF. The human *MIF* gene had 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

References

[1]. Chan, K.; Espinosac , L.; Chaoa, M.; Wongd, D.; Aillesa, L.; Diehna, M. and H.,Gi(2009). "Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells" PNAS ,vol.106 (3),pp. 14016 –14021.

[2]. Akli, S.; Zhang, X.; Bondaruk, J.; Tucker, S., Czerniak, P.; Benedict, F.and K., Keyomarsi(2012)."Low molecular weight cyclin E is associated with p27-resistant, high-grade, high-stage and invasive bladder cancer" Cell Cycle, vol.11(7),pp. 1468-1476.

[3]. Dejong, Y.; Pinckaers, J.; Brinck, R.; Nijeholt, A. and D., OM (2014). "Urinating Standing versus Sitting: Position Is of Influence in Men with Prostate Enlargement. A Systematic Review and Meta-Analysis." PLOSONE,vol.9 (7),pp.e101320.

[4]. Miremami, J. and N., Kyprianou (2014)."The Promise of Novel Molecular Markers in Bladder Cancer". International Journal of Molecular Science, vol. 15(12),pp. 23897–23908.

[5]. Xue, Y.; Xu, H. and L., Rong (2010). "The MIF -173G/C polymorphism and risk of childhood acute lymphoblastic leukemia in a Chinese population" Leukemia Research, vol.34, pp.1282-1286.

[6]. Shimizu,T(2010). "The Role of Macrophage Migration Inhibitory Factor (MIF) in Ultraviolet Radiation-Induced Carcinogenesis" Cancers ,vol.2,pp.1555-1564.

[7]. Spoorthy, N .; Chetal, G. and S., Kumar(2012). "Macrophage Migration Inhibitory Factor: a Potential Marker for Cancer Diagnosis and Therapy". Asian Pacific Journal of Cancer, vol. 13, pp.1737-1744.

[8]. Yuan , T.; Tang , C.; Chen ,M.; Deng,C. and P. ,Chen(2013). "Influence of the human MIF promoter polymorphism on hepatocellular carcinoma prognosis". Genetic and Molecular Research, vol. 12 (4),pp. 6629-6635.

[9]. Meyer-Siegler, K.; Vera, P.; Iczkowski, K.; Bifulco, C.; Lee, A.;Gregersen, P.; Leng, L. and R., Bucala (2007). "Macrophage migration inhibitory factor (MIF) gene polymorphisms are associated withincreased prostate cancer incidence". Genes Immunity, vol. 8,pp.646–652.

[10]. Souza,M.; Curioni , O.; Kanda, J. and M.,De Carvalho (2014)."Serum and salivary macrophage migration inhibitory factor in patients with oral squamous cell carcinoma" .Oncology Letters, vol. 8 (5),pp. 2267-2275.

[11]. Liu, Y. and J., Lin (2014). "Recent advances of cluster of differentiation 74 in cancer". World Journal of Immunology , vol.4(3),pp. 174-184.

[12]. Morris, K.; Nofchissey, R.; Pinchuk, I. and E., Beswick (2014). "Chronic Macrophage Migration Inhibitory Factor Exposure Induces Mesenchymal Epithelial Transition and Promotes Gastric and Colon Cancers". PLOS ONE, vol. 9 (6),pp.1-10.

[13]. Kwok,P and X., Chen(2003). "Detection of Single Nucleotide Polymorphisms". Molecular Biology,vol. 5,pp. 43-60.

[14]. Cardiol, R.(2014)."Genes polymorphism in Human" . Human Gene, vol.22(1),pp.1-12.

[15]. Hosgood, H.; Menashe, I. and M., Shen (2008). "Pathway-based evaluation of 380 candidate genes and lung cancer susceptibility suggests the importance of the cell cycle pathway". Carcinogenesis ,vol.29,pp. 1938–1943.

[16]. Michiels, S.; Laplanche, A. and T.,Boulet (2009). "Genetic polymorphisms in 85 DNA repair genes and bladder cancer risk". Carcinogenesis , vol. 30,pp.763–768.

[17]. Wu, P. and C., Shen(2011)."Hide-then-hit' to explain the importance of genotypic polymorphism of DNA repair genes in determining susceptibility to cancer". Journal of Molecular Cell Biology, vol. 3,pp. 59–65.

[18]. Orita, M.; Iwahana, H.; Kanazawa, H.; Hayashi, K. and T.,Sekiya (1989). "Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms". Proc. Natd. Acad. Sci. USA .,86: 2766-2770.

[19]. Kaur, G and N., Mehra (2012). "Cytokine gene polymorphisms: methods of detection and biological significance". Methods of Molecular Biology, vol. 882 (10),pp.549-568.

[20]. Birdsell, D. N.; Pearson, T.; Price, E. P.; Hornstra, H. M.; Nera, R. D and N., Stone. (2012). "Melt Analysis of Mismatch Amplification Mutation Assays (Melt-MAMA): A Functional Study of a Cost-Effective SNP Genotyping Assay in Bacterial Models". PloS One.,vol. 7(3),pp. e32866-e32872.

[21]. Berno, G.; Zaccarelli, M.; Gori, C.; Tempestilli, M.; Antinori, A.; Perno,L.; Pucill C.; and R., D'Arrigo (2014). "Analysis of single-nucleotide polymorphisms (SNPs) in human CYP3A4 and CYP3A5 genes: potential implications for the metabolism of HIV drugs". BMC Medical Genetics ,vol. 15(76),pp.1-7.

[22]. Yuan, Q.; Meilin, W.; Miaomiao, W•;Zhengdong, Z. and Z . Wei (2012). "Macrophage migration inhibitory factor gene -173G>C polymorphism and risk of bladder cancer in southeast China:a case–control analysis". Molecular Biology,vol. 39:3109–3115.

[23]. Yaun , T.; Tang, C.; Chen, M.; Deng,S. and P., Chen(2013)."Influence of the Human MIF promoter Polymorphism on hepatocellular carcinoma prognosis" Genetics and Molecular Resraech, vol.12(4),pp:6629-6635.

[24]. Das, R.; Subrahmanyan, L.; Ivana, V. ; Duin, D.; Levy, R.; Piecychna, M.; Leng, L.; Ruth ,R.; Shaw, A.; Schwartz, D. and R., Bucala(2014). "Functional Polymorphisms in the Gene Encoding Macrophage Migration Inhibitory Factor Are Associated With Gram-Negative Bacteremia in Older Adults". The Journal of Infectious Diseases ,vol.,209,pp.764–768 .

[25]. Zhang,X.; Weng,W.; Xu,W.; Wang,Y.; Yu,W.;Tang,Y.; Pan,Q.; Wang,J. and F.,Sun(2015). "The association between the migration inhibitory factor −173G/C polymorphism and cancer risk: a meta-analysis" Cancer,vol. 8,pp. 601–613.

[26]. Nishihira,J.;Sato,Y.;Ishibashi,T.;Fukushima,T.;Sun,B.andS.,Todo(2003)."Macrophage Migration Inhibitory factor (MIF)".Ann.N.Y.Acad.Sci.,995:171-182.

[27]. Arikan, C.; Berdeli,A.; Ozgenc, F.; Tumgor, G.; Yagci, R. and S., Aydogdu (2006). "Positive association of macrophage migration inhibitory factor gene-173G/C polymorphism with biliary atresia". J Pediatr Gastroenterol Nutr.,vol. 42,pp.77–82.

[28]. Makhija, R.; Kingsnorth, A. and A., Demaine A (2007)."Gene polymorphisms of the macrophage migration inhibitory factor and acute pancreatitis". Jop., 8:289–295.