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Association between *IL-6* Gene Polymorphism in Position -174 G/C and Bladder Cancer Risk in Iraqi Population

Anwar Abed Nasser Dhabaan[1*]

[1]Al-Iraqia University/ Collage of Education/department of biology, Baghdad, Iraq.
*E-mail: dr.anwar.a.nasser@gmail.com

Abstract:
Our study included two hundred samples of which 120 patients of bladder cancer in Iraqi population, their ages ranged from 30 to 74 year (ages mean 44.83 ± 1.2) and 80 controls (healthy), their ages ranged from 28 to 67 year (ages mean 33.25±1.7). We confined the frequency of *IL-6* Gene polymorphism in position -174 G/C by Tetra ARMS -PCR technique (Tetra-amplification refractory mutation system-polymerase chain reaction technique). Also, we determined the association of *IL-6* Gene polymorphism in position-174 G/C with bladder cancer of Iraqi community. Statistical data showed significant difference in genotype frequency of *IL-6* Gene polymorphism in positoin-174 G/C with patients and control (healthy). The genotype of CC (homozygotes) show high frequency in bladder cancer patients was ratio 50%, comparison with control was 22.50%, and showed association with etiological fraction risk of bladder cancer. The genotypes of GC (Heterozygotes) and CC (Homozygotes) show low frequency in bladder cancer ratio was 24.17% and 25.83% respectively, while in control show high frequency was 48.75% and 28.75% respectively, also GC and CC genotypes showed relationship with preventive fraction (PF) risk of bladder cancer patients. The G allele show high in frequency of bladder cancer patients comparison with control ratio was 62% and 46.88% respectively, and present with etiological fraction risk (EF) of bladder cancer patients in Iraqi population, while C allele show low frequency in bladder cancer patients comparison with control was 37.92% and 53.13% respectively, and present related with preventive fraction (PF) of bladder cancer patients. Our findings demonstrate that the *IL-6* gene in position -174 G/C may represent of bladder cancer risk development of patient's in Iraqi population.
Keywords: *IL-6* Gene, Bladder cancer, Polymorphisms and Tetra ARMS PCR.

1.Introduction

Bladder cancer is the second risk of malignancy in world population [1],also 9th among the most common cancers in world, and comprising about 3% of all malignancies [2], with 430,000 new cases and 165,000 deaths occurring in 2012 [3]. Sex type of males is affected more often than females; as well as the incidence of bladder cancer is increases sharply after the age of 65 year [2]. Some cytokines plays a role in the development of cancer. Several studies have examined the relationship between the interleukin-6 and bladder cancer. Interleukin-6 is protein encoded by gene of *IL-6* and plays a role in response of malignancy, cell survival, proliferation and apoptosis. High level of IL-6 protein in blood serum has been correlated with cancers of which bladder cancer [4], and has correlation between *IL-6* gene in position -174G>C, and risk of bladder cancer [5]. The *IL-6* gene is local on the short arm of chromosome 7 in local 21 of human [6], has 50 single nucleotide polymorphisms (SNP) region in promoter [7]. Among these –174 G/C SNP region (rs1800795) [6] . Variant C/C in *IL-6* gene to G>C in position -174 might be a risk factor for bladder cancer [8]. [9] found that *IL-6* (-174G>C) genotypes are significantly correlated with increasing risk factor of bladder cancer[5], present of *IL-6* -174G>C gene polymorphism significantly relationship with bladder cancer risk of the Asian community. Further studies are needed to validate the risk effect of *IL-6* gene polymorphisms with bladder cancer. The aim of study is to find the

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association between polymorphism of *IL-6* Gene in position (-174 G/C) and risk fraction of bladder cancer for Iraqi population.

2.Materials and Methods

2.1.Study samples

The total of study samples was of 200 samples, of which 120 Bladder cancer patients, there ages range from 30 to 74 year, and 80 controls (healthy), there ages range 28 to 67 year of Iraqi population. All the samples of bladder cancer patients were collected from Oncology Teaching Hospital/Medical City in Baghdad. They had an established diagnosis of bladder cancer by the laboratory (Histological diagnosis) and clinical examination.

2.2.Genotyping of IL-6 Gene in position -174 G/C

Take two ml of blood from each bladder cancer patients and control (healthy) by using venipuncture, later, 2.5 ml was added into EDTA tubes then DNA was extracted by DNA isolation kit (ProMega USA, the according to manufacture instructions manual). DNA purity was qualified by Nano-drop and it was about 1.6 ±1.8. All samples were kept at <22 Cº for further study. *IL-6* gene polymorphism in position (-174 G/C) were examined by using Tetra amplification refractory mutation system-polymerase chain reaction technique (TARMS-PCR). The primers (Alpha DNA-Canada) that designed according to [10] in table (1), and 20 µl were the total volume of reaction mix (PioNeer-Korea), included 5 µl premix master mix (including Taq, dNTP, Buffer, and Mg^{2+}), 1.5 µl of each outer primer and 1.5 µl of each inner primer, and 5 µl DNA, and 4 µl of RNase-free double distilled water), and the molecular marker size (Pro-Mega-USA) 100-1500 base pair. Tetra ARMS-PCR programs of *IL-6* gene polymorphism in position -174 G/C was summarized in table (2), according to [10]. The genotypes were established by analyzing electrophoresed 2% gel of agarose stained with diamond dye (Pro-Mega).

Table 1. Primer sequences of *IL-6* gene in position (-174 G/C) by TARMS -PCR technique.

The studied Gene	primer	Sequences of primer (5`→ 3`)	Size (bp)
IL-6 -174 G/C gene	F Outer	GACTTC AGCTTT ACTCTTTGTCAAGACA	326 bp
	R Outer	GAATGAGCCTCAGACATCTCCAGTCCTA	
	F inner (G allele)	GCACTT TTCCCC CTAGTTGTGTCTTCCG	205 bp
	R inner (C allele)	ATTGTGCAATGTGACGTCCTTTAGCTTG	184 bp

Table 2. The program *IL-6* (-174 G/C) gene polymorphism by using TARMS PCR technique for bladder cancer and control samples.

Target gene	Steps	Temperature (c^o)	Number of cycles	Time (seconds)
IL-6 -174 G/C gene	Pre-denaturation	95		600
	Initial denaturation	95		30
	Annealing	54	40	30
	Extension	72		30
	Final Extension	72		600

2.3.Statistics

Differences in the frequencies of *IL-6* gene in position -174 G/C for bladder cancer in this study with control were analyzed by Fisher's test (value P<0.05). The OR (Odds Ratios) and

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CI (Confidence Intervals) were calculated by Compare 2 Ver.3.04 software Abramson (2003-2013). The Preventive Fraction (PF) and Etiologic Fraction (EF) results were compared with Hardy-Weinberg equilibrium and according to the software within the following website www.had2know.com.

3. Results

The study of genetic polymorphisms for *IL-6* gene in position -174 G/C for 120 bladder cancer patients was ages mean 44.83 ± 1.2 year, and 80 control (healthy) samples was ages mean 33.25±1.7 year. Notably, the two alleles G/A are more present for *IL-6* gene polymorphism in position -174 G/C with GG, GA and AA genotypes in bladder cancer patients and control (healthy) (Figure 1), by Tetra ARMS -PCR technique. The frequency distribution of genotypes polymorphism showed significance in bladder cancer comparison with control samples by Fisher's test (value P>0.05). The genotyping polymorphisms frequency of IL-6 -174 G/C gene was significance in bladder cancer patients, so GG genotype show significant and high frequency in bladder cancer patients compared with control, and it was 50% and 22.50% respectively (Figure 1), also the OR of GG genotype was 3.44, and CI was 1.83 to 6.48 (table 3). The GG genotype present association with etiological fraction for bladder cancer risk ratio was 35.5%, while GC genotype present low frequency in bladder cancer patients compared with control was 24.17% and 48.75% respectively (Figure 2), also the OR was 0.34 and CI was 0.18 to 0.61, and show relationship with protective fraction (PF) of bladder cancer risk ratio was 32.4%. The value of GC genotype as protective fraction (PF) was 0.65 (table 3), As well as CC genotype showed high frequency in control compared with bladder cancer patients was 25.83% and 28.75% respectively, also the OR was 0.65 and CI was 0.35 to 1.19, and present relationship with protective fraction (PF) of bladder cancer risk ratio was 3.9%. Briefly, the results showed that GG genotype was correlated etiological fraction (E. F) with the risk of bladder cancer in Iraqi population, while GC and CC genotypes were correlated with the protective fraction (PF) of bladder cancer in Iraqi Population.

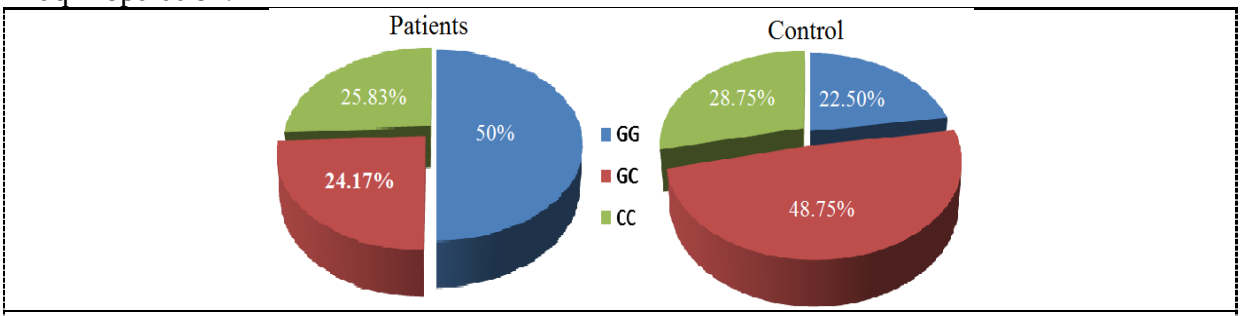

Figure 1. The frequencies of IL-6-174 G/C genotypes in bladder cancer and control samples.

The G and C allelic was different in frequency of bladder cancer patient compared with control (healthy), so G allele show high frequency in bladder cancer patient comparison with control was 62.08% and 46.88% respectively (Figure 2), while OR (1.86) and CI (1.24 to 2.78), and it was 28.6% as an etiological fraction (E. F) are presented in table (3), while C allele show low frequency in bladder cancer comparison with control was 37.92% and 53.13% respectively (Figure 2), while OR (0.54) and CI (0.36 to 0.83), and it was 24.5% as an protective fraction (P. F) are presented in table (3). The G allele was significance in bladder cancer patients comparison with control was *0.002 (P<0.05 by Fisher's test). Briefly, polymorphism of IL-6 -174 G/C gene show that G allele is an etiological fraction and it's describe that the C allele be a preventive fraction that correlated with bladder cancer risk.

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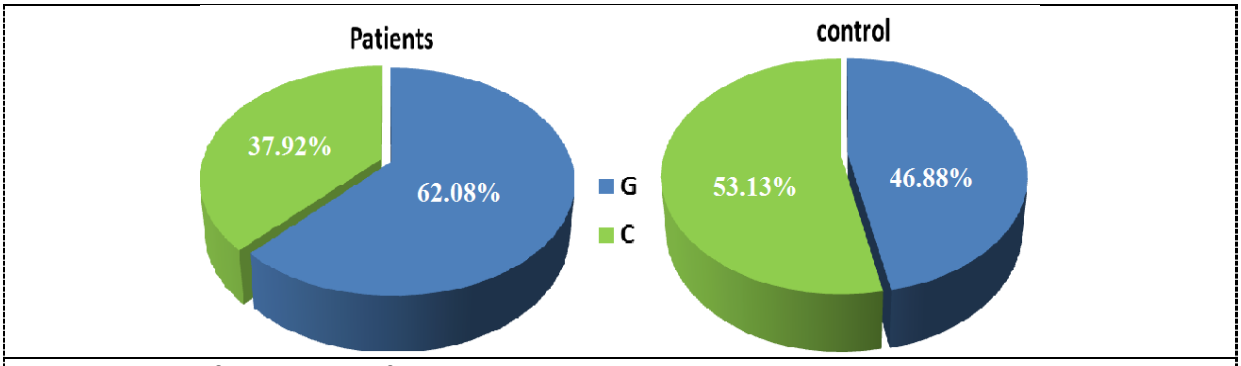

Figure 2. The frequencies of IL-6 -174 G/C allelic in bladder cancer and control samples.

Table 3. The frequency of allelic and genotypes in bladder cancer and control samples for of *IL-6* Gene in position (-174 G/C).

Gene	Genotype & Allele	Bladder cancer(%)Number	Healthy (%)Number	OR (CI 95%)	P. value
IL-6 -174 G/C gene	GG	60 (50%)	18 (22.50%)	3.44(1.83 to 6.48)	*
	E. F		35.5%		0.000
	GC	29 (24.17%)	39 (48.75%)	0.34 (0.18 to 0.61)	*0.000
	P. F		32.4%		
	CC	31 (25.83%)	23 (28.75%)	0.65 (0.35 to 1.19)	0.109
	P. F		3.9%		
	G allele	149(62.08%)	75(46.88)	1.86(1.24 to 2.78)	
	E. F	28.65%			*0.002
	C allele	91 (37.92%)	85(53.13%)	0.54(0.36 to	
	P. F	24.5%			

Notes: OR (Odds ratio), CI (Confidence Interval), E.F (Etiological fraction), P.F (Preventive fraction), P<0.05 by Fisher's.

4. Discussion

Interleukin-6 is a major inflammatory cytokine that plays important roles in genetic and immune responses [9] (Nader et al., 2014). IL-6 is pleiotropic cytokine increase risk of infection and development of some tumor types of which bladder cancer, including bladder cancer [11]. Most SNP of region -174G/C (single nucleotide polymorphism) of *IL-6* gene influence on binding of the receptor and thus repress transcriptional activation of inflammatory proteins [12], that IL-6 in position (-174 C>G) genotype and allele is significantly correlated with increased risk fraction in bladder cancer [9], and studies have found an relationship between IL-6 and bladder cancer risk. [13] found that variation of (C/C) genotype for *IL-6* gene was correlated with increasing of risk fraction of bladder cancer, while, genomic data did not reveal an association between bladder cancer patients and control in genotypes frequency of *IL-6* gene polymorphism in postion174G>C in patients [14], while [9], suggests that IL-6 in position (-174 C>G) genotypes are present correlated with risk development of bladder cancer. The (C/C) genotype of IL6 gene has beneficial effect for Bladder cancer-treated patients [8]. However, one Indian study showed that *IL-6* gene in position (-174G>C) have protective fraction against bladder cancer [15]. The current study showed a relationship between the GG genotype of IL-6 in region (-174 C>G) and risk development with bladder cancer in Iraqi population.

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5. Conclusion

The statistical data of bladder study demonstrate the polymorphism association of IL-6 -174 G/C gene with risk of bladder cancer, and indicate that the *IL-6* gene in position -174 G/C may represent a significant risk factor in patients of bladder cancer of Iraqi population..

6.References

[1] Sinha R, Anderson DE, McDonald SS and Greenwald P 2003 Cancer risk and diet in India*J. Postgrad. Med.***49** 222.
[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, and Bray F 2015 Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN*Int. J. Cancer***136** E359.
[3] Prasad V and Goldstein JA 2014 US News and World Report cancer hospital rankings: do they reflect measures of research productivity. *PLoS One***9** e107803.
[4] Grivennikov S and Karin M. 2008 Autocrine IL-6 signaling: a key event in tumorigenesis? *Cancer Cell*. **13** 7.
[5] Chiang Y, Chen-Hsun H, Su-Wei H, Tse-Yen Y, Chih-Wei S, Yuan-Hung W and Chia-Chang W 2018 Association between the rs1800795G>C polymorphism in the promoter of interleukin-6 gene and bladder cancer*Int. J. Clin. Exp. Pathol.***11** 3598.
[6] Capurso C, Solfrizzi V, D'Introno A, Colacicco AM, Capurso SA, Capurso A and Panza F 2004 Interleukin 6-174 G/C promoter gene polymorphism and sporadic Alzheimer's disease: Geographic allele and genotype variations in Europe*Exp Gerontol.***39** 1567.
[7] Pereira DS, Garcia DM, Narciso FM, Santos ML, Dias JM, Queiroz BZ, Souza ER, Nóbrega OT and Pereira LS 2011 Effects of 174 G/C polymorphism in the promoter region of the interleukin-6 gene on plasma IL-6 levels and muscle strength in elderly women*Braz. J. Med. Biol. Res*. **44** 123.
[8] Ahirwar D, Kesarwani P, Manchanda PK, Mandhani A and Mittal RD 2008 Anti-and proinflammatory cytokine gene polymorphism and genetic predisposition: association with smoking, tumor stage and grade, and bacillus Calmette-Guerin immunotherapy in bladder cancer. *Cancer Genet. Cytogenet*. **184** 1.
[9] Ebadi N, Jahed M, Mivehchi M, Majidizadeh T, Asgary M and Hosseini SA 2014 Interleukin-12 and interleukin-6 gene polymorphisms and risk of bladder cancer in the Iranian population*Asian Pac. J. Cancer Prev*. **15** 7869.
[10]Mehrabadi A, Dor MK, Taybeh B and Simin H 2018 Analysis of Polymorphism and Expression Profile of ASIC and IL-6 Genes in Patients with Gastric Cancer. *Asian Pac. J. Cancer Prev*. **19** 3451.
[11]Chen MF, Lin PY and Wu CF 2013 IL-6 expression regulates tumorigenicity and correlates with prognosis in bladder cancer*PLoS One***8** e61901.
[12]Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S and Woo P 1998 The effect of novel polymorphisms in the interleukin-6 (*IL-6*) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis*J. Clin. Invest*. **102** 1369.
[13]Leibovici D, Grossman HB, Dinney CP, Millikan RE, Lerner S, Wang Y, Gu J, Dong Q and Wu X 2005 Polymorphisms in inflammation genes and bladder cancer: from initiation to recurrence, progression, and survival*J. Clin. Oncol.* **23** 5746.
[14]Zhang H, Xu Y and Li L 2012The interleukin-6 -174G/C polymorphism and prostate cancer risk: a systematic review and meta-analysis. *Urol Int.***88** 447.
[15]Gautam KA, Muktanand T, Sankhwar SN, Goel A, Sankhwar PL and Rajender S 2016 Functional polymorphisms in the IL6 gene promoter and the risk of urinary bladder cancer in India *Cytokine***77** 152.