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THE EFFECT OF RS9642880 G > T ON THE INCIDENCE OF BLADDER CANCER IN BABYLON PROVINCE

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ABSTRACT

Background: Bladder cancer (BC) is a multifaceted disease accompanying with high morbidity and mortality rates if not treated optimally.

multifaceted disease accompanying with increase rate of death if not cured is Bladder cancer (BC).

Aims: this study aimed to investigate the association between (rs9642880 G > T) single nucleotide polymorphism (SNP) in the *Myc* gene and the incidence of BC.

Subjects and Methods: one hundred BC subjects and 100 healthy subjects (control group) were participated in this revision. Blood sample were taken from all participant for DNA extraction. Specific primers were used for amplification of *Myc* gene by conventional polymerase chain reaction (PCR). Restriction fragment length polymorphism (RFLP) were used for SNPs Genotyping.

Results: Results of (rs9642880 G>T) SNP of *Myc* gene failed to indicate significant (p >0.05) association with the occurrence of BC under genotype distribution, inheritance models and minor allele frequency analyses.

Conclusion: The *Myc* gene SNP (rs9642880 G>T) positively associated with the development of BC in Babylon province.

Key words: Bladder cancer, single nucleotide polymorphism, *Myc* gene

1. INTRODUCTION

Worldwide, the seventh most frequently type of cancer in male is Bladder cancer, while considered as the eleventh most common type of cancer in both gender.

According to the archive of Iraqi Cancer Registry, BC characterizes the communal type of malignancy in Iraq [(1)]. BC considered more dangerous for females than males because of increase rate of mortality, in addition female has advanced stage when diagnosis and had a propensity to be older age **[(2)]**.

Many factor like smoking **[(3)]**, industrial contact to chemicals **[(4)]**, genetic factor **[(5)]**, radiation [(6)], bladder infections for a long time, subject with age 55 years or more and the incidence of Bladder cancer in men is more than women **[(7)]**.

The gene encodes a nuclear phosphoprotein for intracellular processes and act as proto-oncogene transcriptional factor is *Myc* gene **[(8)]**.

The family of *Myc* oncogene involves three members, C- *Myc*, *Myc*N, and *Myc*L, which encode c-*Myc*, N-*Myc*, and L-*Myc* respectively.

The *Myc* oncoproteins is the member of a family called "super-transcription factors" which control the transcription of about 15% of the entire genome [(9)].

In the human gemome, C- *Myc* is located on band q24.21 of chromosome 8 and consist of three exons and two introns [(10)].

The members of *Myc* family display high-structural homology, including the basic-region /helix-loop-helix/leucine-zipper (BR/HLH/LZ) at the C terminus and three highly preserved elements, identified as Myc boxes 1–3 at the N terminus. Wide range of cellular functions is regulate by *Myc* gene. Protein -coding or non-coding genes that involved in different functions of cell (are regulated by *Myc)*, like cell cycle, cell adhesion, protein biogenesis, signal transduction, metabolism, transcription, and translation **[(11)]**.

More than 25% of tumors, in different kind of cancer like breast, colorectal, pancreatic, gastric, and uterine cancer, *Myc* genes are the most commonly deregulated oncogene [(12)].

Impaired *Myc* expression in cancers happens through gene amplification, chromosomal translocation, focal enhancer amplification, germline enhancer polymorphism or, through constitutive activation of upstream signaling pathways **[(13)]**.

The present study was designated the potential association between bladder cancer and *Myc* polymorphism in Babylon province.

2. SUBJECTS AND METHODS

Subjects

These revision include 100 subjects with bladder cancer (clinically diagnosis depend on pathological stage of the urinary bladder), and 100 healthy controls with age range (38-79 years old).

This study was carried out on patients attended to Babylon oncology center in Merjan Medical City, Hilla city, Babylon province, Iraq.

For DNA extraction 2 milliliters of venous blood was occupied in EDTA tubes (for all participant) then kept at -20 until be used.

DNA extraction and genotyping of *Myc* gene

The DNA was extracted from the venous blood by genomic DNA mini kit (Favorgen, Taiwan) . the SNP (rs9642880 G>T) was amplified by specific primer similar to Mei Zhao, *et al*. 2018 **[(14)]**, as shown in table 1.

Table (1) :The forward and reverse primers for *Myc* gene SNP with their product size.

Markers	Primers	Primer sequence 5'-3'	Product size	Enzyme
rs9642880	F	5'-CCACCACTCTCAGCCTTTTC-3'	203 bp	StyI-HF
	R	5'-TGGGATTACAAGTGTGAACCTG-3'		

Maxim PCR Pre-Mix Kit was used for amplification of *Myc* gene, (2 µL) of DNA from each sample and (0.25 µL) of primers were added to each master-mix tube (20 µL) PCR Master Premix kit , Promega(USA). The master-mix tubes (after mixing), were transferred to the PCR Thermo cycler (Techno / UK). The program that gave the best results of amplification of *Myc* Gene (rs9642880 G>T) SNP include, 4 min at 95 ºC for initial denaturation , followed by 30 sec of denaturation at 95 ºC for (35 cycles) , 30 sec for annealing at 57ºC, 30 sec for extension at 72 ºC , and 5 min for final extension at 72 ºC.

The (rs9642880 G > T) SNP of *Myc* gene was evaluated by the restriction enzyme StyI-HF, one band (203 bp) represent the wild genotype (GG) whereas the two bands (135 and 68 bp) represent the homozygotes genotype (TT) and the heterozygotes genotype (GT) produced three bands (203, 135, and 68 bp) [(14)].

In RFLP the protocol and chemicals used in restriction digestion reaction of *Myc* gene rs9642880 G > T SNP that gave the best results include: 1.0 µL restriction enzyme was added to 5 µL of PCR product then 10 µL of dilution buffer and of nuclease free water 34 µL were added then incubated for 6 hr at 37°C. 2% agarose gel electrophoresis were used for the investigated of digestion products.

Agrose gel electrophoresis

The digested product of the restriction enzyme was assessed by agarose gels electrophoresis(according to Harisha method) [15].

The molecular weight of the DNA, agarose concentration, voltage applied and strength of the electrophoresis buffer will determine the rate of migration or mobility in the gel through the electric field. Red-Safe nucleic acid staining solution was used for direct determination of DNA location within the gel, then gel was applied to UV light and the photos was taken [16].

Statistical Analysis

SPSS (statistical package for social science) version 20 was approved for Statistical analysis.

Genotyping and allele frequency were expressed. Hardy Weinberg equilibrium (HWE) characterize the mathematical relationship between genotypes and allele frequencies [17]. Chi-squared test were used for HWE measurement by the comparison between observed genotypes with expected values[18].

The relationship between patients genetic patterns and control was examine using the Odd ratio (OR) with 95% confidence interval (CI). p value ≤ 0.05 considered Statistically significant.

3. RESULTS

The amplification of *Myc* gene exhibited an amplicon of 203 bp. It contained the target SNP (rs9642880 G > T), figure (1). The digestion of the product by a restriction enzyme explored three patterns ,figure(2):

- 203 bp is the wild genotype (GG), represent one band.
- 135 and 68 bp are the homozygous genotype (TT), represent two bands.
- 203, 135 and 68 bp are heterozygous genotype (GT), represent three bands.

Genotyping frequencies of rs9642880 G > T of gene polymorphism were indicate to be consistent with Hardy-Weniberg equilibrium HWE (p > 0.05) in bladder cancer patients and the controls group, as shown in table (2).

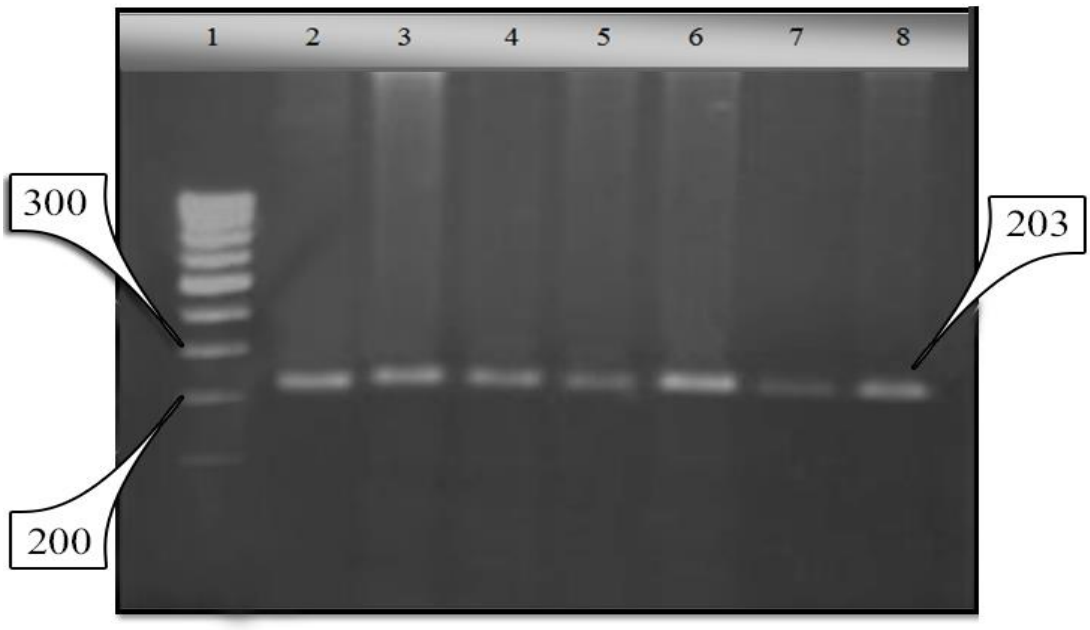
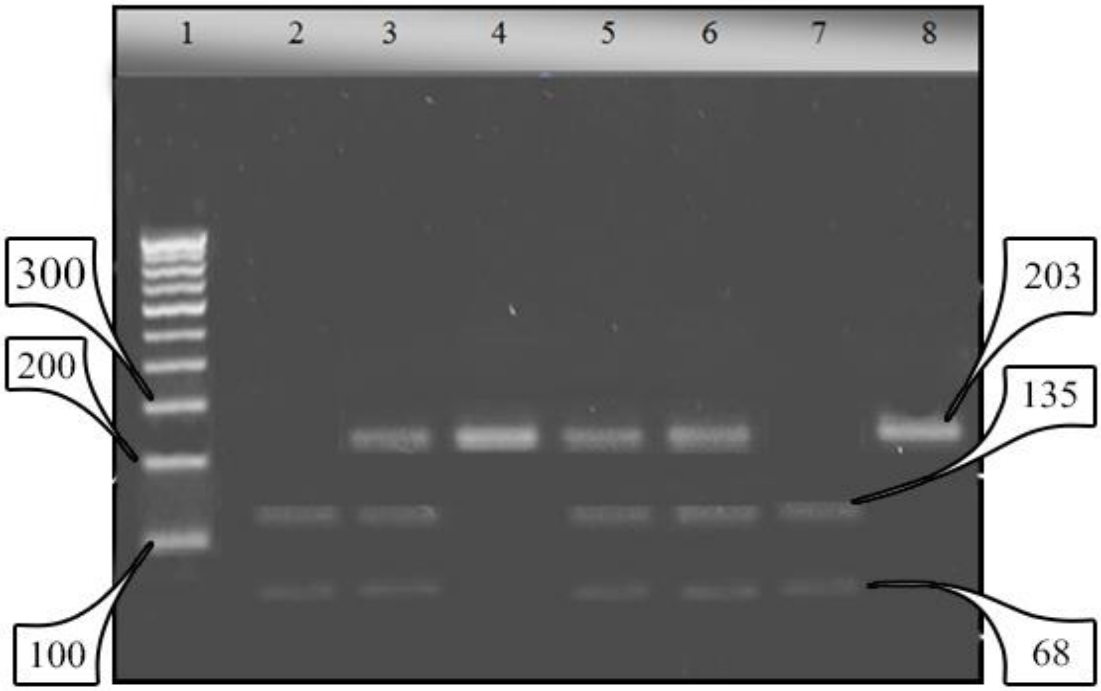


Figure (1): The PCR Product of *Myc* Gene (rs9642880 G>T) SNP in 2% Agarose Gel Electrophoresis. Lane 1: Ladder. Lane 2-8: PCR product (203 bp)



Figure(2): Restriction Digestion of PCR Product of *Myc* Gene (rs9642880 G>T) SNP in 2% Agarose Gel Electrophoresis.

- Lane 1: Ladder.
- Lane 4 and 8: 203 bp are wild genotype (GG).
- Lane 3,5 and 6: 203, 135 and 68 bp are heterozygous genotype (GT).
- Lane 2 and 7: 135 and 68 bp are homozygous genotype (TT).

Table (2): Results of Hardy Weinberg Equilibrium for *Myc* Gene (rs9642880) SNP genotypes in Bladder cancer patients and Controls Groups

Group	X^2	P-value
Control	0.226	0.633
Patient	0.034	0.851

The association of each genotype with bladder cancer was further tested under different models of inheritance as depicted in table (3) . Results of rs9642880 G>T SNP of *Myc* gene failed to indicate significant (p >0.05) association with the occurrence of BC under genotype distribution, inheritance models and minor allele frequency analyses.

Table (3) : Association of rs9642880G>T genotypes with bladder cancer under different models of inheritance

Model	Genotype	Control	Patients	OR (95%CI)	P-value
Codominant	GG	50 (50%)	38 (38%)	1	_
	GT	40 (40%)	48 (48%)	1.57 (0.87-2.86)	0.132
	TT	10 (10%)	14 (14%)	1.84 (0.73-4.59)	0.19
Dominant	GG	50 (50%)	38 (38%)	1	0.088

	GT-TT	50 (50%)	62 (62%)	1.63 (0.92-2.86)	
Recessive	GG-GT	90 (90%)	86 (86%)	1	0.386
	TT	10 (10%)	14 (14%)	1.46 (0.61-3.47)	
Over dominant	GG-TT	60 (60%)	52 (52%)	1	0.255
	GT	40 (40%)	48 (48%)	1.38 (0.79-2.42)	

O.R.:Odds Ratio; non-significant at p > 0.05

The allele distribution and frequency of *Myc* gene (rs9642880 G > T) SNP shown in Table (4). The allele frequencies of G and T of *Myc* gene (rs9642880 G > T) SNP were found to be 62% and 38% in BC patients respectively and 70% and 30% in the control group respectively. The minor allele frequencies (T) of *Myc* gene (rs9642880 G > T) SNP in BC patients and control groups were found to be 38% and 30% respectively. It was non-significantly change (P > 0.05) in BC patients when compared with that of the controls group.

Table (4) Alleles Distribution and Frequency of *Myc* Gene (rs9642880 G > T) SNP in Bladder cancer Patients and Controls Groups

Allele		Control	Patient	OR (95% CI)	P-value
G	No.(%)	140 (70%)	124 (62%)	Reference	Reference
T	No.(%)	60 (30%)	76 (38%)	1.43(0.94-2.16)	0.091

O.R.:Odds Ratio; non-significant at p > 0.05

4. DISCUSSION

Several genetic polymorphisms of *Myc* gene have been identified. One of the most common polymorphism of the *Myc* gene is (rs9642880) SNP positioned in the LD block directly adjacent to the c-*Myc* oncogene and about 30kb upstream of it [(19)].

The heterozygous genotype (GT) and homozygous genotype (TT) of *Myc* gene (rs9642880 G > T) SNP were found to be non-significantly increase (OR=1.57, CI 95% 0.87-2.86, P=0.132) and (OR=1.84, CI 95% 0.73-4.59, P=0.19) respectively the risk of BC with respect to those of the wild genotype (GG) of *Myc* gene (rs9642880 G > T) SNP. The current results are in consistence with the results of : Kiemeney LA, *et al*. (2008) **(20)** in which no difference was observed in the *Myc* Gene (rs9642880) SNP among BC and control groups. While our result are in contradiction with several previous study like Wang M, *et al* (2009) **(21)**, Ali S, *et al* (2013) **(22)** and Tang J, *et al* (2015)**(23)** showed that the T allele of SNP rs9642880 confers liability to bladder cancer in Chinese, Pakistani, Asian and Caucasian populations.

Conclusion

The rs9642880 G > T SNP of Myc gene was studied previously in other population and showing the direct association of allele T in the prevalence of bladder cancer.

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