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Genetic Association between Human DNA Double-Strand Break Repair Gene Xrcc7 Polymorphism with Staging and Development of Bladder Cancer

Saleh Ali Al-Qadoori[1*], Hedef Dhafir El-Yassin[1], Usama Sulaiman Al-Nasiri[2]
[1]*Department of Clinical Biochemistry, College of Medicine, University of Baghdad, Baghdad*
[2]*Iraqi Council of Medica Specializations, Baghdad, Iraq.*

Abstract

Urinary Bladder Cancer is the 6th most common malignancy in males worldwide, and the second in Iraq. DNA Double-Strand Break Repair Gene polymorphism may cause a reduction in DNA repair capacity and influence an individual's susceptibility to bladder cancer and the prognosis of the disease. In the present study, we attempted to investigate the influence of active tobacco smoking and human DNA repair gene XRCC7 (rs7003908) polymorphism on bladder cancer risk. Also, the impact of XRCC7 polymorphism on the staging and development of the disease. A total of 62 of histo-pathologically confirmed diagnosed bladder cancer patients, and 38 age-matched healthy controls were involved in the study. All were recruited from February to September 2017 in a case-control study conducted in the Department of Biochemistry at the College of Medicine University of Baghdad. Genotyping of the XRCC7 polymorphism (G>T) was evaluated using a polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) and confirmed by Sanger sequencing method. The study found that patients with the homo polymorphic gene (GG genotype) have significantly increased the risk of bladder cancer (OR, 4.0; $p = 0.03$). While subjects having the homo wild-type gene (TT genotype) could decrease the risk of bladder cancer. G Allele genotypes were also observed to be associated with a significantly increased risk of T2 (OR, 3.7; $p = 0.001$), and for Ta (OR, 3.5; $p = 0.003$). A statistically highly significant increased bladder cancer risk in the smoker with G Allele (OR, 5.5; $p = 0.0001$). The study suggests that having polymorphic gene GG genotype of DNA Repair Gene XRCC7 could increase the risk of bladder cancer and also affect the development and staging of the disease while having the TT genotype could decrease the risk of bladder cancer and improve the survival rate of bladder cancer patients.

Keywords: Bladder Cancer (BC), XRCC7, Polymorphism, rs7003908, DNA Double-Strand Break Repair (DSBs).

Introduction

Polymorphisms in DNA Double-strand repair genes are wildly being studied for an increased bladder cancer risk (1). These genes have an important role in maintaining genome integrity (2). Alter in in DNA Double-strand repair mechanism (DSBs) can cause chromosomal abnormalities which in turn can progress to cell malfunctioning, cell apoptosis, and tumorigenesis (3). Recently, many studies have established that DNA Double-strand repair genes polymorphisms were a responsible modifier of many cancer and diseases risk (4). Studies show that Different environmental and occupational factors, such as chemical carcinogens, including aromatic amines, aromatic hydrocarbons, and N-nitroso compounds, reactive oxygen species and many anticancer drugs are causes in the development of urinary bladder cancer (5). However, not all individuals exposed to these carcinogenic factors developed cancers, which suggesting an individual's susceptibility to exposure-gene related carcinogenesis (6). The higher risk factors of bladder cancer are Tobacco smoking which is a carcinogenic component contained a wide variety of chemicals such as reactive oxygen species, nicotine, sugars, minerals, and proteins, are naturally-occurring in tobacco leaves. In the time of cigarette combustion both the chemicals which exist naturally in the tobacco and the new chemicals which are formed at the time of the combustion are released into the tobacco smoke, and all could induce tumor forming (7). Recently studies show proved that reactive oxygen species in tobacco smoking can induce double-strand DNA base damage (8). DSBs cause chromosomal instability and leading to the development of cancer (9). Endogenous or exogenous sources of Double-Strand Breaks can be repaired by a variety of mechanisms, each with a different possible mutagenic consequence. Non-homologous end-joining (NHEJ) is the major DSBs repair mechanism (10). Canonical NHEJ pathway, repair is largely regulated by two complexes (11). The first complex, a heterodimer of KU70 and KU80 (KU70/80), is important for the capture, stabilization, and processing of broken ends (12). The second complex, composed of DNA ligase IV (LIG4) and its cofactor XRCC4, rejoins the processed ends (13). Although KU70, KU80, LIG4, and XRCC4 comprise the heart of the C-NHEJ pathway (13). The initial step in C-NHEJ is binding of the broken ends by KU70/80 (14). With its toroidal structure, KU70/80 can dynamically bind a variety of duplex DNA ends including blunt ends, 5' overhangs, 3' overhangs, and hairpin loops (15). Upon binding a DNA end, KU70/80 serves as a support for the recruitment and assembly of several other important repair proteins (16). In the final steps of C-NHEJ, the LIG4/XRCC4 complex functions to ligate the DNA termini (16). DNA Dependent Protein Kinase abbreviated DNA-PK is a holoenzyme of three subunits, Ku70, Ku80, and DNA-Pk catalytic subunit(15) . This kinase plays important roles in DNA double-strand break (DSB) as shown in figure 1 (15). According to the GenBank accession no: NM_001469, The human XRCC7 is a Non-homologous end-joining double-strand break repair gene (17). XRCC7 encodes the catalytic subunit of DNA-activated protein kinase (DNA-PKcs), which involved in the NHEJ repair pathway (18). A very recent study finds out that Deficiencies in DNA-PK activity are clinically significant (19). Inactivation components of DNA-PK in Mice show severe combined immunodeficiency as well as ionizing radiation hypersensitivity (20). Genetic variation G6721T (rs7003908) of XRCC7 (rs.7003908) is located in the intron 8 of the promoter KU70 gene region KU70(17) . Wang and his colleagues find that this that this polymorphism, may regulate splicing and cause mRNA instability (21). However, a few studies on the G6721T (rs7003908) polymorphism of XRCC7 associated with several types of cancers have been published with conflicting results (17). XRCC76721G > T Genetic variation is located in an intron region and could lead disturbing splicing mechanism cause mRNA instability which in turn can develop cancer (22). XRCC76721G > T polymorphisms are linked with an increased risk of many cancers, including glioma, prostate, and breast cancer, respectively (22) (21) (22) (23) (24). Moreover, studies revealed that there was no significant link between the XRCC7 rs7003908 polymorphism and risk of renal cell carcinoma and differentiated thyroid cancer (25). Despite our knowledge, The association of XRCC7 rs7003908 polymorphism and the impact of smoking on the bladder cancer risk and staging is not studied yet. We hypothesized that smoking plus the polymorphic type XRCC7 rs7003908 polymorphism are contributed to the etiology and the staging of bladder cancer. To

test this hypothesis, we genotyped XRCC7 rs7003908 polymorphism in a case-control study of bladder cancer patients in the Iraqi population.

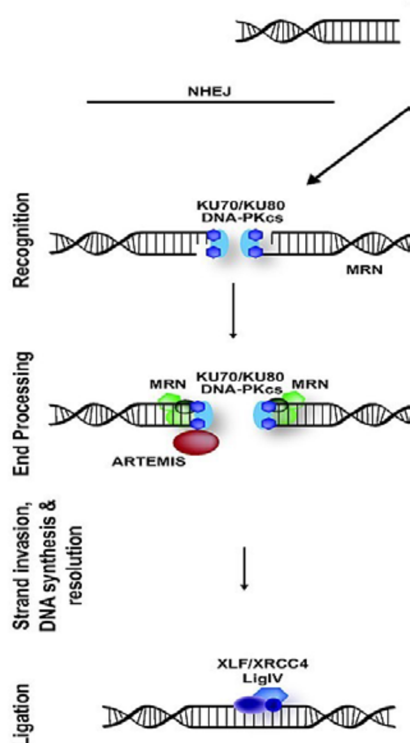

Figure 1:Non-homologous end joining (NHEJ) in mammals during DNA double-strand break.

MATERIALS AND METHODS.

PATIENT AND CONTROL SAMPLE

A Case-control study conducted at the Chemistry and Biochemistry Department University of Baghdad/college of medicine, this study was performed on 100 subjects during the period from February 2017 to September 2017. All patients were recruited from Gazi Al-Harery Hospital for Specialized Surgery/ Baghdad/ Iraq. Out of these 100 subjects, 62 subjects (47 males, 15 females) with urinary bladder cancer and 38 cancer-free subjects (28 males, ten female). The participants in this study were age and sex match. A mean age (± SD) for bladder cancer was 63.6±8.3 years and mean age (± SD) 63±6.5 years for healthy controls. All patients were first diagnosed with bladder tumor and investigated by a urologist and underwent cystoscopy examination for transurethral resection of bladder tumor (TURB) or undergo cystoscopy with biopsy of bladder lesion for histopathological examination. The main exclusion criteria were: subjects with a history of urinary tract infection, bladder stone, a patient with previous cancer, with cancer metastasized to bladder from another origin and those with previous chemotherapy or radiotherapy. Control subjects were cancer-free and had no history of tumors, and were recruited from the patient's companion. Subjects who smoked once a day for more than six months were defined as ever smokers. After taking authorization agreement from the subjects a Five mL Whole Blood samples were obtained into sterile EDTA tubes and stored at -4°C for genomic DNA extraction.

DNA EXTRACTION AND GENOTYPING

Promega DNA extraction kit (USA) was used in the Genomic DNA extraction from was extracted from whole blood Samples, which were collected in 5ml tubes containing ethylenediaminetetraacetic acid (K3EDTA) from bladder cancer cases and free-cancer controls. Extracted DNA was stored at −80°C for further SNP genotyping. XRCC7 6721G>T Fragments amplified using polymerase chain reaction (PCR). Primers for the genotyping of the DNAPK XRCC7 gene Fragments was newly designed using multiple primer design software by NCBI and Sigma Aldrich. Primer sequences were 5′-CGGCTGCCAACGTTCTTTC -3′ (forward) and 5′-GTGGTTCCCTGGTGCTCAAT-3′ (reverse) were used to amplify the target fragment containing the XRCC7 6721G>T polymorphism. The fragments of the XRCC7 6721G>T polymorphism were amplified in 25 mL of reaction mixture containing 2 µl of genomic DNA template, 0.75 µl of each primer, 9 µl H2O, 12.5 µl of PCR master mix (Promega, Madison,WI, USA) which contain 0.1 mM of each dNTP, 1 ¥ PCR buffer, 10 mM Tris-HCl, 50 mM KCl and 0.1%Triton X-100), 1.5 mM MgCl2, and 1.0 unit of Taq polymerase.

The PCR amplification program was as follows: one cycle for 4-min as denaturation step at 95°C; 30 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 3 min. The PCR product was 360bp and was checked on a 2% agarose gel as shown in figure 2. PCR product was digested with the restriction enzyme of PvuII (New England Biolabs, Beverly, MA, USA). PCR product and PvuII were incubated at 37°C for 24 hours and then separated on a 3% agarose gel stained with ethidium bromide which is shown in figure 3. The PvuII cut site is CAG_^CTG which cut in the wild-type T site of the XRCC7 6721G>T producing 2 fragments (266 bp, 94 bp) for the homozygous wild-genotype, 3 fragment (360 bp, 266 bp, 94 bp) of heterozygous genotype and 1 fragment (360 bp) for the homozygous polymorphic genotype. For confirming this results, 10% of the 360 bp PCR products samples with three different genotypes were randomly selected and confirmed by direct sequencing using by Sanger sequencing method (Macrogen, South Korea) as shown in figure 4.

STATISTICAL ANALYSIS

The data of the study were stored in a Microsoft Excel spreadsheet and analyzed on the computer using the SPSS software 16 and Microsoft excel program (2016). Numeric variables were expressed as mean ± SD. Student t-test was used for comparison of mean between two groups. Chi-square test used to compare frequency. Chi-square test was performed to evaluate differences in frequency distributions of demographic characteristics, certain variables, and each genotype and allele of the XRCC7 6721G>T polymorphisms between the bladder cancer cases and free- cancer controls. Moreover, we determine if the cases and control samples were demonstrated Hardy–Weinberg equilibrium. Unconditional univariate and multivariate logistic regression analyses were carried out to calculate ORs and 95% CI and to obtain the association of bladder cancer risk with the genetic polymorphisms of XRCC7 and for the joint effects of cigarette smoking and staging of the bladder cancer.

RESULTS

CHARACTERISTIC OF THE SUBJECTS

Total of 100 individuals was analyzed in the study in the present study. The control groups consisted of 38 healthy individuals, while the patients were 62. Demographic characteristics of the voluntary groups are summarized in Table 1. Patients and control was sex, age, weight, height, and BMI matched. Mean ± SD was calculated for the two group (patients and control). There was no statically different association between bladder cancer patients and control p-value > 0.0 for this parameter.

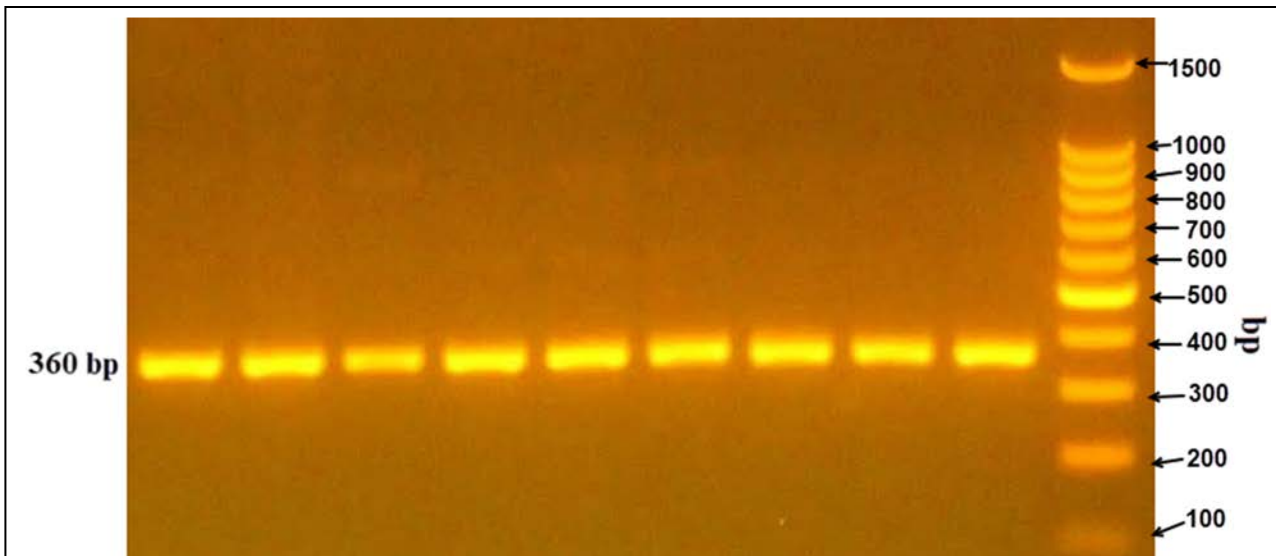

Figure 2: An electrophoretic graph of the PCR product of the XRCC7 rs7003908 Gene polymorphism using Promega master mix on 1% agarose, 70V, and for 2 hour (7 µl of DNA loaded in each well).

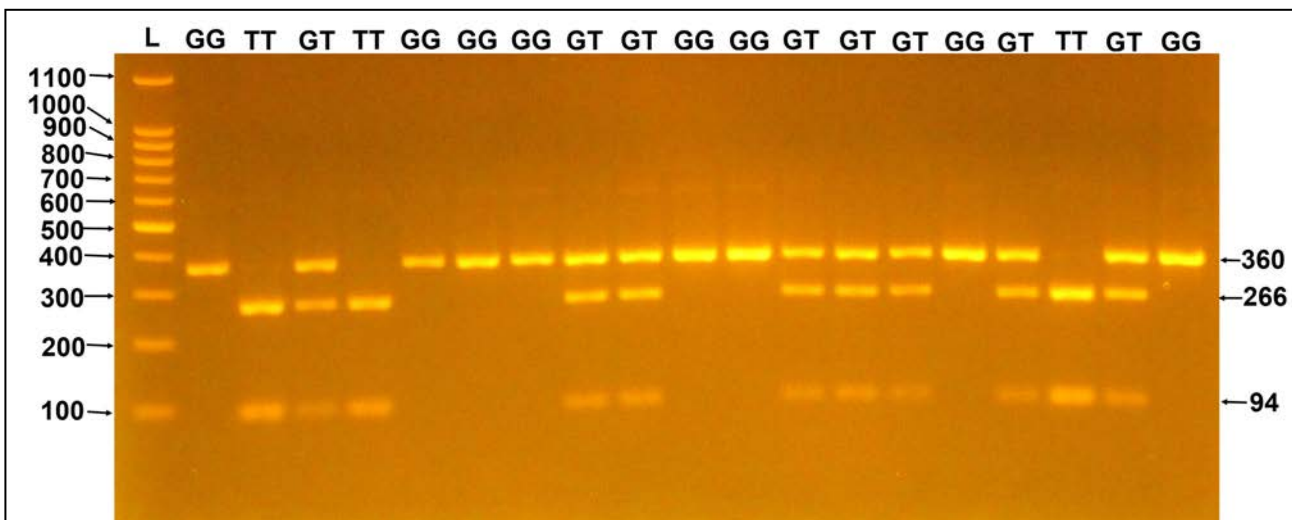

Figure 3: An electrophoretic graph of the PCR product after digestion with the restriction enzyme (PvuII). The electrophoretic graph containing various genotypes of the XRCC7 G > T.

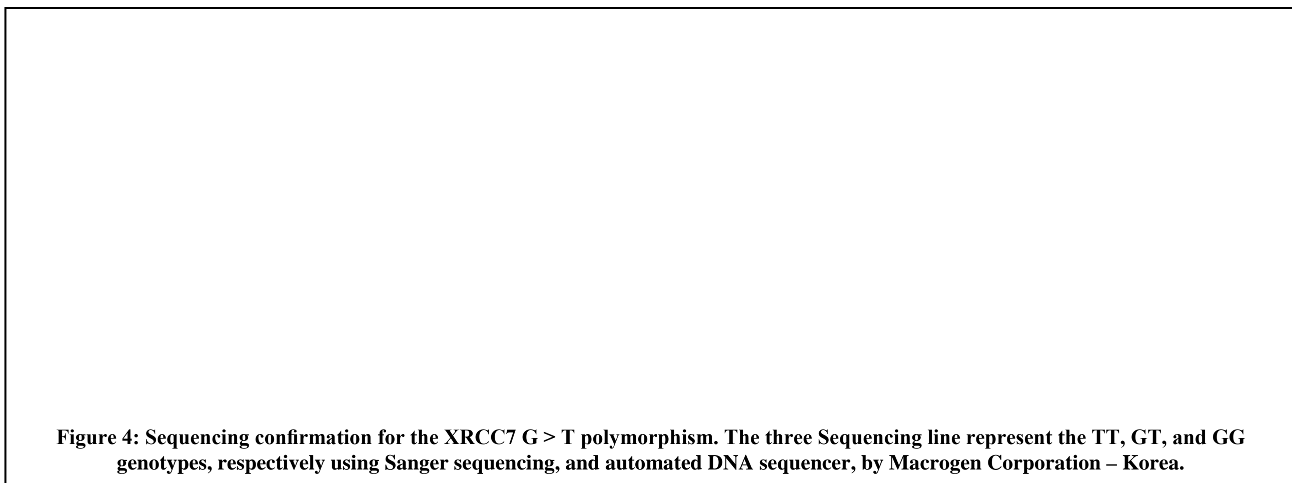

Figure 4: Sequencing confirmation for the XRCC7 G > T polymorphism. The three Sequencing line represent the TT, GT, and GG genotypes, respectively using Sanger sequencing, and automated DNA sequencer, by Macrogen Corporation – Korea.

Table 1: Basic characteristics of study groups.

Categories	Cases n=68 (mean ± SD)	Control n=38 (mean ± SD)	*p*
Age	63.6±8.3	63±6.5	0.38
weight	80.5±10.3	82.4±11.2	0.09
Height	170.7±9.5	167.9±6.9	0.07
BMI	27.8±4.2	29.3±3.9	0.06

Table 2: Frequency distributions of selected variables between the bladder cancer cases and cancer-free controls

		Cases (n = 62)		Controls (n = 38)			
Variables		Count	N%	Count	N%	OR (95% CI)	*p*†
Sex	Female	15	24.2%	10	26.3%	1.00	-
	Male	47	75.8%	28	73.7%	1.11 (0.44-2.82)	0.81
Age group (years)	>60	39	62.9%	26	68.4%	1.00	-
	<=60	23	37.1%	12	31.6%	1.2 (0.54-3.00)	0.57
Smoking status	Never-Smoker	19	30.6%	20	52.6%	1.00	-
	Ever-Smoker	43	69.4%	18	47.4%	2.51 (1.09-5.79)	0.03*
Cancer Stage	Ta	17	27.4%	-	-	-	-
	T1	26	41.9%	-	-	-	-
	T2	19	30.6%	-	-	-	-

Table 3: Genotypes and Allele frequency of XRCC7 rs7003908 among bladder cancer and control.

XRCC7	Genotype, n (%)			Allele frequency		(HWE) *p-value*
	TT	GT	GG	p	q	
Bladder Cancer	12 (19.4)	34 (54.8)	16 (25.8)	0.47	0.53	0.43
Control	15 (39.5)	18 (47.4)	5 (13.2)	0.63	0.37	0.92

Table 4: Distribution/genotyping of XRCC7 rs7003908 polymorphism in 68 Bladder Cancer patients and 38 cancer-free controls.

	Cases (n = 68)		Controls (n = 38)			
XRCC7	Count	N %	Count	N %	OR (95% CI)	p
TT	12	19.4%	15	39.5%	1.00	-
GT	34	54.8%	18	47.4%	2.3 (0.91-6.1)	0.07
GG	16	25.8%	5	13.2%	4.0 (1.13-14.08)	0.03*
GT+TT	50	80.6%	23	60.5%	2.7 (1.09-6.72)	0.03*
T	58	46.75%	48	63.15%	1.00	-
G	66	53.25%	28	36.85%	1.95 (1.08-3.49)	0.02*

Table 5: Impact of Smoking on XRCC7 rs7003908 Allele polymorphism in cases and controls.

Smoking status	Genotype	Count	n %	Count	n %	OR (95% CI)	p
non-smoker	T	14	36.8%	26	65.0%	1.00	-
	G	24	63.2%	14	35.0%	3.1 (1.2-8.0)	0.01*
smoker	T	44	51.2%	22	61.1%	3.7 (1.6-8.4)	0.001**
	G	42	48.8%	14	38.9%	5.5 (2.2-13.5)	0.0001***

Table 6: Association of DNA repair gene XRCC7 rs7003908 polymorphism with tumor stage categories.

	Control	Bladder Cancer Stage			(a-b)			(a-c)			(a-d)		
XRCC7	(a)	Ta(b)	T1(c)	T2+(d)	OR	95 % CI	P	OR	95 % CI	p	OR	95 % CI	p
TT	15 (39.5)	0 (0)	9 (34.6)	3 (15.8)	1.00								
GG+GT	23 (60.5)	17 (100)	17 (65.4)	16 (84.2)	23	1.2-421	0.03*	1.2	0.43-3.4	0.69	3.4	0.8-14	0.07
T	48 (63.2)	11 (32.4)	35 (67.3)	12 (31.6)	1.00								
G	28 (36.8)	23 (67.6)	17 (32.7)	26 (68.4)	3.58	1.5-8.4	0.003**	0.8	0.39-1.7	0.6	3.7	1.6-8.4	0.001**

Male was more frequent to have bladder cancer than female (75% of individuals were male) table 2. The highest number and percentage of patients with bladder cancer were found to be at the age of>60 years which is showed in table 2. Smoker individuals showed a high risk of bladder cancer comparing to the non-smoker group OR (95% CI) 2.51 (1.09-5.79) p 0.03. Bladder cancer Patients were grouped according to the stage of cancer to 3 group (Ta, T1, T2). In this study T1 was the highest frequent stage among the three stages showed in table 2.

Genotypes and Allele frequencies for XRCC7 rs7003908

XRCC7 rs7003908 polymorphism distribution and allele frequencies in the cases and bladder cancer groups and the results of Hardy– Weinberg equilibrium are shown in table 3. The polymorphic allele frequency of the XRCC7 rs7003908 in bladder cancer patients was higher than in control (0.53, 0.37) respectively. The polymorphic GG genotype in the bladder cancer group was 25.8% of total bladder cancer patients, while in 13.2% in the control group. For the XRCC7 rs7003908, both bladder cancer and control group was in Hardy– Weinberg equilibrium p-value >0.05.

Comparison of XRCC7 rs7003908 polymorphism.

Using multivariate logistic regression, odds ratios were calculated by taking the homozygous wild-type (TT) as reference genotype and comparing the rest genotypes with it (heterozygous GT genotype, homozygous polymorphic genotype GG genotype, and GT+GG) as shown in table 4. Homozygous polymorphic GG genotype showed a highly significant increase in bladder cancer risk OR (95% CI) = 4 (1.13-14.8), p=0.03. However, no significant association was seen in the Study subjects who carried the GT genotype OR (95% CI) = 2.3 (0.91-6.1), p=0.07. Also, there was a high statically significant increase in GG+TT genotype of the XRCC7 rs7003908 and bladder cancer risk compared to the wild-type TT genotype OR (95% CI) =2.7 (1.09-6.72), p=0.03. Moreover, the polymorphic G allele genotype of the XRCC7 rs7003908 showed a statically significant increase in bladder cancer risk when compared with the wild-type T allele genotype OR (95% CI) = 1.95 (1.08-3.49), p=0.02.

Association of XRCC7 rs7003908 Polymorphism with smoking status

To measure the combined effect of XRCC7 rs7003908 the wildtype T allele genotype in never-smoker individuals was taken as reference genotype and are shown in table 5. Polymorphic allele G genotype of XRCC7 rs7003908 showed high significant association bladder cancer risk among in non-smoker individuals OR (95% CI) = 3.1 (1.2-8.0), p=0.01. For smoker individuals, Both T and G allele showed a significant increase in bladder cancer risk p-value was (p=0.001 for T genotype and 0.0001 for the G genotype). However, there was 48.64% increase in the risk of the bladder cancer Odd ratio of the G allele from the T allele (OR for T allele=3.7, OR for G allele= 5.5).

Association XRCC7 rs7003908 genotypes with bladder cancer stage

Patients were stratified into three Categories according to the stage of bladder cancer (low stage Ta, medium T1, higher stage T2). The odds ratio was measured by comparing the three-stage genotypes with the control genotypes study. The wild-type TT genotype was taken as reference and compared with the GG+GT genotype as shown in table 6. The XPC rs2228000 GG+GT genotype showed a highly significant increase in Ta (OR=23, p=0.03). However, GG+GT genotype did not show any statistically significant association in increased the risk of bladder cancer for the T1 or T2 stages of bladder cancer (p=0.69, 0.07) respectively.

When comparing the wildtype T allele with polymorphic genotype T allele of the XRCC7 rs7003908, G allele showed highly significant increased risk of bladder cancer for the higher stages of bladder cancer T2 with OR of 3.4 and p-value of 0.001. Also, a statically significant association has been found for the lower stage Ta of the bladder cancer and the polymorphic allele G of the XRCC7 rs7003908 polymorphism OR=3.58, p=0.003. Moreover, no statically significant association was found for the T1 stage of bladder cancer and the polymorphic G allele of the XRCC7 rs7003908 polymorphism p>0.05.

Discussion

The polymorphisms of XRCC7 gene had been associated with the risk of many cancers including the bladder cancer; however, the results were inconvenient. To make the result more accurate, different ethnic group is needed to study, and the Iraqi population has the best gene pool that was not studied yet. Higher polymorphism allele frequency was found among bladder cancer patient (q=0.53) comparing to polymorphic allele frequency in the control group (q=0.37). In another hand, the wild-type allele frequency was higher in the control group (p=0.63) and lower in bladder cancer (p=0.47). Both study groups were in Hardy– Weinberg equilibrium p-value >0.05, and that made the pool of study individuals suitable for this genetic study. The disruption of the T and G allele of XRCC7 rs7003908 is highly variable among different population, however, the T allele was higher in all studied population, the higher polymorphic allele frequency was seen among south Asian population with q frequency of 0.443 against the wild-type with p frequency equal to 0.557 (26, 27). The nearest polymorphic q allele frequency to our population for the XRCC7 rs7003908 was seen in East Asian with q allele frequency of 0.631 and p allele frequency of 0.369 (26, 27). Interest in recent years has concentrated on the population-wide variability in XRCC7 repair capacity phenotypes, which appears to account for a several-fold variation in cancer risk (28-30). In the present study, an association was found in the homozygous polymorphism GG genotype with increase bladder cancer risk with an OR of 4 when compared to the wild-type TT genotype. Also, the highly significant association was found to increase bladder cancer risk was when comparing the GG+GT genotype with the same wild-type GG genotype of the XRCC7 rs7003908 polymorphism. However, the GT genotype of the XRCC7 rs7003908 showed no statically significant association to increase bladder cancer risk, but the p-value was near the significant value (0.07). In the previous study, GG and GT+GG of the XRCC7 rs7003908 polymorphism showed highly significant association in increase bladder cancer risk among Indian population (p=0.001) (31). Moreover, GT genotype of XRCC7 rs7003908 polymorphism also showed significant association with increased bladder cancer risk p=0.029. However, different in the p-value of the GT genotype of the XRCC7 rs7003908 and increase bladder cancer risk value is negligible. Similarly, a statically significant association was found between the XRCC7 rs7003908 polymorphism and increased bladder cancer risk among southern Chinese population (32). In this study, the polymorphic allele G genotype showed a highly statically significant association in increase bladder risk with OR equal to 0.02. This finding is compatible with many previous studies (27, 30-33). This indicated that XRCC7 is associated with bladder cancer risk with a different population. The mechanism of this increase is not fully discovered. Double-strand breaks in DNA are the most dangerous class of DNA damage because they may lead to either cell death or loss of genetic material and unrepaired can result in genetic instability and ultimately may enhance the rate of cancer development (30). Also, DNA damage is one of the major

reasons that cause many tumors (34). Moreover, DNA damages are corrected by DNA repairs mechanisms (35). Damages to the double strand DNA repair by the Non-homologous end-joining (NHEJ) which is the major DSBs repair mechanism(36). Mutation in the genes that encoding the units of the NHEJ such as the DNA-Pk catalytic subunit which is one of the important units of the NHEJ repair pathway can disrupt the protease protein structure confirmation; which lowers its activity (37). One of the major cancer causes is the accumulation of DNA damage because cells lose the ability to control its growth (38). DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis (39). Furthermore, the polymorphic G allele showed high statically and significantly association of increasing bladder cancer risk in both smoker and never-smoker individuals. However, smoker individuals odd ration was higher than never-smoker approximately by a half percent. This indicates that genetic factor with the presences of the occupational factor could highly increase bladder cancer risk. Similar interaction of the XRCC7 polymorphism with the smoking status was previously found in the different population. In the previous study among Indian population, Current smoker carrying with the XRCC7 polymorphism showed a statically significant increase in bladder cancer by OR of 3.22 and p=0.004. A recent study showed that DNA Double-strand mechanism deficiency in mice increases cell susceptibility to cell death through apoptosis during cigarette smoke exposure (40). There are several studies and evidence on the DNA repair mechanism that support our findings (25, 32, 33). Mutation in the genes responsible for double-strand DNA repair mechanism plus smoking may result in the accumulation of genetic defects and affect the instability and ultimately which enhance the rate of cancer development. Compounds in smoke, such as 4-(N-Methylnitrosoamino)-1-(3-pyridyl)-1butanone (NNK) and N'-nitrosonornicotine (NNN), can cause DNA damaging events in the urothelium. Also, more than seventy chemicals are known to cause, initiate or promote cancer (41, 42). Smoking with the absence of DNA repair mechanism enhances cellular proliferation and may enhance urinary bladder carcinogenesis (43). This finding point to the fact that Smoking with the absence of a DNA double-strand mechanism, could highly affect the structure of the DNA and enhanced tumor development and metastasis such as bladder cancer.

Furthermore, the polymorphic allele G of the XRCC7 rs7003908 polymorphism showed a highly significant association with Ta and T2 stages of bladder cancer risk (p=0.03 for Ta, p=0.001 for T2). Also, the highly significant association was found in bladder cancer stage Ta in individuals carrying the (GG+GT) compared with individuals caring the wild-type TT genotype (p=0.03). A similar finding has been found in the north Indian population; GG genotype showed an increased risk for Ta and T1 p=0.001 and T2 0.001 OR=6.8 (31). The rate of developing of the cancers cells is increased proportionally to lowering of the immunity (44). Recently studies show that KU70 may function as a porter gene for the development of T-cell lymphomas. Deficiency or lowering the activity of the KU70 may lower the development of the T-cell lymphomas and decrease the immunity (45, 46). This is supporting the finding that XRCC7 could increase the prognosis of the bladder cancer and the genetic study of the XRCC7 could be a useful tool to tackle these emerging problems and is an attractive target for individuals anticancer treatment.

In summary, the current study observed that the XRCC7 rs7003908 polymorphism independently increased the susceptibility of bladder cancer in the Iraqi population. More importantly, the combined influence of smoking and mutant gene enhance the formation of bladder cancer tumor. Also, it has been found that the rate of developing and prognosis of the disease is profoundly affected by the mutation of the XRCC gene which made the genetic marker powerful in the diagnosis and monitoring Bladder cancer tumors. Cohort-study and further structural-functional analysis are needed to evaluate the biological mechanism of this polymorphism XRCC7 and the bladder cancer risk.

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