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No Association Between of ctla-4 Gene Polymorphism (-1722T/C) and Bladder Cancer in Patients from South Iran

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ABSTRACT

Introduction: The CTLA-4 molecule is expressed in subset T cell. This is a key regulator of the T cell immune response. It is established that blockade of CTLA-4 receptors leads to the enhancement of an immune response. Different polymorphisms of the CTLA-4 gene have been described which cause increased susceptibility to various malignancies such as lymphoma or multiple myeloma In this study we analyzed single nucleotide polymorphism (SNP) at position -1722T/C ctla-4 molecule in patient with bladder cancer and compare with normal subject. Materials and Methods: the ctla-4 -1722T/C, polymorphism was studied in 185(157 male + 28 female) patient with bladder cancer and 173 (120 male +30 female) healthy subject by using Restriction fragment length polymorphism (PCR-RFLP) and data were compared in both groups by using Pearson's chi-square test. Results: The results indicated that there is no significant difference in the frequency of genotypes and alleles of ctla-4 gene at position (-1722T/C) in patients with bladder cancer and the control group (Pv>0.05). Also there was no correlation between the frequency of genotypes and alleles with the clinicopathological factors in the patients. Discussion: our study showed no relationship between ctla-4 gene polymorphism at -1722T/C position and genetic susceptibility to the development of bladder cancer.

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INTRODUCTION

Today, cancer is one of the fundamental problems of modern societies. The cytotoxic T lymphocyte -4 Molecules is expressed on the surface of activated CD4+ and CD8+ T lymphocytes and T regulatory cells. [1] CTLA4 protein is a homodimer molecule , have a disulfide bond in the extracellular domain at cysteine residue 120.This glycoprotein molecule is structurally similar to CD28 and binds to the B7 isoforms , CD80 (B7.1) or CD86 (B7.2) , on the cell surface of antigen presenting cells with an affinity that is 10 to 100 times that of CD28 . However, unlike CD28, this molecule may inhibit lymphocyte activity[2].This molecule plays an important role in the maintenance of peripheral tolerance as one of the key negative regulators of an acquired immune response. .[3, 4] CTLA-4 molecule may inhibit T-cell responses by antagonizing CD28-CD80 mediated signals.

T-cells are known for their important antitumor immunity role and therefore molecules such as CTLA-4 that mediate regulation of T-cell activity could affect cancer susceptibility.[5] Studies showed that antibodies against this molecule, mediate cancer regression and autoimmunity in patients suffering from renal cancer and metastatic melanoma due to increased T-cell activation.[6]

About 100 single nucleotide polymorphisms in the ctla-4 gene region is known, six of them are more important than others. These six positions are +1822C/T,-1661A/G, +49A/G,-1722T/C,-318C/T and -658C/T. [7]

Many studied showed the relation between Ctla-4 gene polymorphisms and abnormalities, such as oral squamous cell carcinoma, type 1 diabetes, non melanoma skin cancer, breast cancer, human papilloma virus 16 related cervical cancers and renal cell carcinoma . [8,9]

Considering the importance of gene polymorphism in the expression of the CTLA-4 and the role of genetic factors in cancer development, In this case-control study we aim to evaluate the one SNPs of CTLA-4 gene polymorphism at position -1722T/C and its susceptibility to bladder cancer

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MATERIALS AND METHODS

Patient and control groups:

This study was carried out from February 2011 to sep 2013. The patient group consisted of 185 individuals with a mean age of 48.2± 11.6 who were selected from those who presented with symptoms of bladder cancer, such as gross hematuria. Their diagnosis of bladder cancer was later confirmed by cystoscopy and their bladder cancer had been confirmed by pathological studies There were 173 age and sex match normal with an average age of 50.8±11.8, healthy individuals selected as the control group. All patients were consented prior to participating in the study. Each of the subjects from both groups had 5cc of blood drawn and stored at 4ºC in an EDTA tube for DNA extraction (10).

Genotyping:

Previously reported polymerase chain reaction/restriction fragment length polymorphism methods were used for allelic determinations Promoter-region polymorphisms at positions –1722 was genotyped as follows by using a single primer set: forward, 5′ CTAAGA GCA TCC GCT TGC ACC T 3′, reverse 5′ TTG GTG TGATGC ACA GAA GCC TTT T 3′ (11). PCR was performed on a total volume of 25 µL containing 1.5 µL of 1 u/µl DNA Taq polymerase(Cinna Gene, Iran), 1 µL of each primer (12.5 picomolar) and 1 µg of genomic DNA (0.3 µg/µL). gene Amplification was performed with initial denaturation at 94ºC (3 min), followed by thirty-five cycles at 94ºC (40 s), 70ºC (40 s), 72ºC (40 s), and a final extension at 72ºC (5 min), resulting in a product of 486 bp. Amplified fragments were digested with BbvI (BsexI)(Fermentas, Canada) at 65ºC for 3 h. digestion of the amplified 486 bp product with this enzyme resulted in 270 bp and 216 bp fragments. Products were visualized on a 2% agarose gel by using KBc Power load (Fig1).

Statistical analysis:

The results were compared between the case and control groups according to the age of onset, history of cigarette smoking, tumor size, grade and local or distant metastases. The data were analyzed using the statistical program SPSS 15 (IBM, USA) and EPI Info 2000 (Georgia,USA). Chi squire (x^2) and Fisher's exact tests were applied to determine the differences in genotype/alleles frequencies. Arlequin 3.1 software package (12) was used to determine if the distribution of genotypes are in Hardy Weinberg equilibrium. A p-value of < 0.05 was considered statistically significant.

Results:

In this study, the gene polymorphisms at position -1722T/C in *ctla-4* gene was studied among 192 patients with Bladder cancer and 215 women as controls. Table 1 shows the demographic data of the subjects in the case group. The prevalence of TT, TC and CC genotypes at position -1722T/C according to the PCR-RFLP method was 66%, 32.9% and 1.1% in the patient group, respectively. While the prevalence of these genotypes in the control group was: TT (65.3%), TC (32.4%) and CC (2.3%). Statistical analysis indicated that there is no significant difference between the distribution of various genotypes with bladder cancer and control group (P=0.44). There was no significant relationship between this genotype and the presence of bladder cancer or its stage as compared to the control group (Table 2). There was also no significant difference between the prevalence of the T allele or C allele at this position in both the case and control groups (Table 3).No significant correlation was found between the frequency of inherited genotype and and clinical-pathological factors of the disease including tumor size, mean age of cancer onset and demographic data of the patients as well as risk factors; all of which revealed no association (Table 4).

Discussion:

CTLA-4 is a negative regulator of the B_7 family that is the main ligand of CD28. On the other hand CTLA-4/ CD80 interactions block activation and promote T-cell death or apoptosis (11–14) Recent studies suggesting that CTLA-4 deficiency induces or exacerbates autoimmunity, enhances tumor immunity or prevents stimulation of immunologic tolerance. [9] Stimulated T-cells are covered up in lymph nodes mainly by ephemeral expression of the CTLA-4 molecule on their own surface after rendezvous by B_7 on APCs. Kato suggested that CTLA-4 engagement promotes T-helper 1 rather than T-helper 2 differentiation. [12]

In contrast, it is known that immunologic tolerance and anergy allows tumors like bladder cancer to prevaricate the immune response. One of the main management protocols for superficial bladder cancer has been the intravesical administration of Bacille-Calmette-Guérin (BCG) that causes inauguration of T-cells. [7, 15] thus, it can be postulated that factors such as different genetic polymorphisms that escort to suppression or decreased role of T-cells could play a function in tumor genesis in bladder cancer. Li et al investigated the JWA gene promoter polymorphism in 215 patients with bladder cancer and fulfilled that the -76C allele and 454A allele of this gene were both associated with a notably augmented risk of bladder cancer. [17]

Bid et al showed that interleukin-1Ra gene polymorphism was uncommonly present in patients with bladder cancer and discussed the key role that interleukin 1Ra gene polymorphism plays in bladder cancer development. [18]

Association of CTLA-4 polymorphism in different types of cancers has been well-known; Erfani et al. confirmed that variations in CTLA-4 promoters are important in development of breast cancer. However, they confirmed that this polymorphism does not play a role in the progress of breast cancer [19]. whereas no association between CTLA-4 polymorphism (AG genotype) and colorectal adenoma could be recognized in a study involving 132 colorectal cancer and 186 colorectal adenoma patients in an Italian population,[20] another investigation revealed that this type of polymorphism (AG genotype) was actually accompanied by an increased risk of colorectal cancer in the Chinese population [21]. One study confirmed the role of CTLA-4 polymorphism in augmented susceptibility to multiple myeloma[22] .

Although association of the CTLA-4 gene with some of the urinary system malignancies such as RCC is well established[5], according to our information no such study has been performed on bladder cancer awaiting now. Our data showed no correlation between CTLA-4 genotypes at position 49A/G and bladder cancer and no relationship between the invasiveness of bladder cancer and CTLA-4 gene polymorphism at this position.

It seems in bladder cancer more restricted factors rather than systemic ones, such as gene polymorphism, might contribute to the pathogenesis. In fact, inhibition of T-cell role as a result of CD28 receptor activation on T-cells by CTLA-4 might not be the key mechanism occupied in the tumor genesis of bladder cancer. The role of other factors such as defects in humoral immunity, natural killer cell defects and further tumorogenic factors must be investigated.

In conclusion, our data shows no relationship between CTLA-4 polymorphism and genetic susceptibility to the augment of bladder cancer and no association with disease development. Further studies should be performed in both Iranian and other populations in classify to have more conclusive results.

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Table 1: Demographic characteristics of patients with bladder cancer.

Demographic characteristics	N=185(%)
Sex:	
• Male	157 (84.8%)
• Female	17 (15%)
Age (mean±SD)	62.3±6.8
Age of onset of disease (mean±SD)	61.64±2.3
History of smoking	82 (51.8%)
Tumor characteristics*	
• Low grade tumor	50 (46%)
• High grade tumor	59 (54%)
• Mean tumor size (cm)	3.38
• Distant metastasis	12 (7.5%)
• Local metastasis	43 (23.2%)
Treatment protocol*	
• TURB**	90 (48.6%)
• Radical cystectomy	15 (8.1%)
• TURB followed by radical cystectomy	64 (34.6%)
• Radiotherapy	
• BCG therapy***	17 (9.1%)
• Chemotherapy	19 (10.2%)
	22 (11.8%)

* In these categories, data are combined together from different aspects. Therefore the sum of the data might surpass 100% due to an overlap between data (such as treatment protocols) in the subgroups.**TURB: Transurethral Resection of Bladder, ***BCG: Bacille-Calmette-Guérin

Table 2: Frequency of different CTLA-4 genotypes in the patients and control groups.

Type of polymorphism				Total (n=358)
	Patient group (n=185)	Control group (n=173)	P value	
TT genotype (%)	122 (66%)	113 (65.3%)	0.401	235 (65.6%)
TC genotype (%)	61 (32.9%)	56 (32.4%)	0.404	117 (32.7%)
CC genotype (%)	2 (1.1%)	4 (2.3%)	0.409	6 (1.7%)

Table 3: Frequency of different alleles of CTLA-4 gene in both patient and control groups.

Groups	"T" allele frequency %	"C" allele frequency %	P value
Patient	305 (82.4%)	65 (17.6%)	
Control	282 (81.5%)	64 (18.5%)	
Total	587 (81.9%)	129 (18.1%)	0.587

Table 4: Comparison of different CTLA-4 genotypes and their association with tumor characteristics.

	Age of onset (mean)	Tumor size (mean)
TT genotype	67.23	3.66
TC genotype	58.42	5.10
CC genotype	62.11	4.64
P value	0.72	0.31

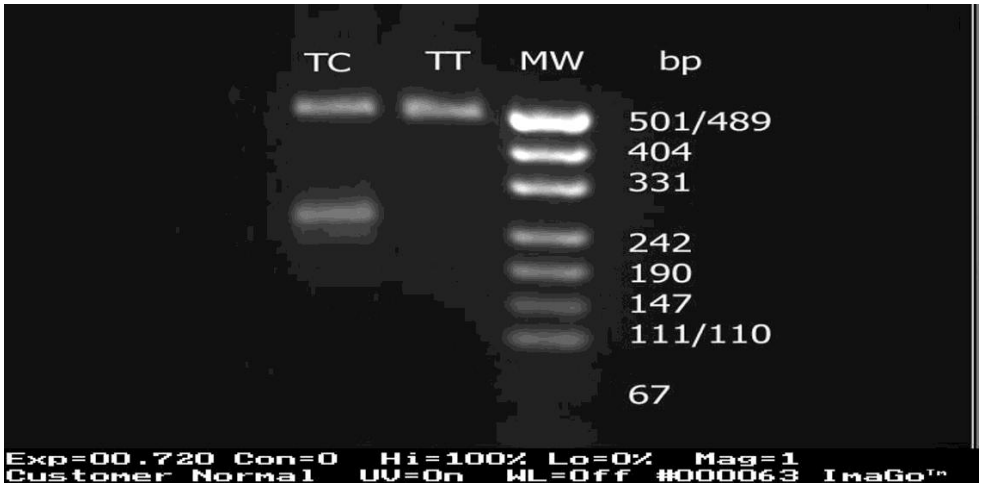

Fig. 1: PCR restriction fragment length polymorphism results of -1722 T to C substitution in *CTLA-4* promoter region.

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