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Genetic Polymorphism of Stromal Cell-Derived Factor-1 Gene in Iraqi Patients with Urinary Bladder Carcinoma

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Abstract

The aim of the study was to investigate the association between the *SDF-1 rs1801157* polymorphism, has a G > A transition in the 3′-UTR and serum concentrations of SDF-1 which might contribute in cancer susceptibility to Urinary Bladder Carcinoma (UBC). Sixty patients have been diagnosed with UBC and 40 healthy controls. The results of the serum SDF-1 estimated by using ELISA technique revealed a significant increase in UBC patients when compared to the healthy group, also there is a significant increase in the high stages and invasion of the diseases. SDF-1 genetic polymorphism (rs1801157) SNP analysis by sequencing showed that results have a good comparison with the equilibrium of Hardy-Weinberg. The frequency of GG showed higher frequency compared to control with no significant positive Odd ratio (OR) (1.74) with confidence interval (CI= 0.72 - 4.18, P = 0.28). While the AG genotype showed a significantly lower frequency in patients (55.5%) with a significant negative OR (0.41) with (CI= 0.18 - 0.92, P=0.03) and the Preventive fraction was (Pf=0.32).

Keywords: Genetic Polymorphism; Gene; in Iraqi Patients;

INTRODUCTION

The bladder is a balloon-shaped organ in the pelvic area that stores urine. Bladder cancer is the most common cancer of the urinary tract with ~380,000 new cases and ~150,000 deaths per year worldwide. It ranks fifth among cancers in men in Western countries. Bladder cancer typically affects older adults, though it can occur at any age (1). Most patients present with haematuria (blood in urine), and diagnosis is made following cystoscopy and biopsy. Majority of bladder cancers cases are diagnosed at an early stage which is highly treatable; however, even early-stage bladder cancer is likely to recur. Therefore, bladder cancer survivors often undergo follow-up tests for years after treatment to look for bladder cancer recurrence (2). Bladder cancer begins most often in the cells that line the inside of the bladder. Currently, there are two classification systems in use. At diagnosis, the majority of bladder cancers (~60%) are in-muscle invasive (stage Ta) papillary tumors of low grade (3).

Almost 70% of bladder tumors are non-muscle invasive tumors (stage Tis, Ta, or T1), 25% are muscle invasive (stage T2 or T3), and 5% are metastatic. Metastasis is the main cause of death. Many requirements are required for metastasis to occur, including, cell wall attachment, neovascularization, invasion, and cell proliferation. Cell adhesion molecules (CAMs) play important roles in cell-cell and cell-matrix interactions. these criteria associated with invasion and metastasis in a large variety of human malignancies including urothelial cancers.

Stromal cell-derived factor-1 (SDF-1), which now is designated as CXCL12, is a homeostatic chemokine that signals through CXCR4, which in turn plays an important role in hematopoiesis, development, and organization of the immune system (4).

CXCL12 secretion by stromal cells attracts cancer cells, acting through its cognate receptor, CXCR4, which is expressed by both hematopoietic and nonhematopoietic tumor cells. CXCR4 promotes tumor progression through direct and indirect mechanisms. First, CXCR4 is essential for metastatic spread to organs where CXCL12 is expressed and thereby allows tumor cells to access cellular niches, such as the marrow, that favor tumor-cell survival, and growth. Second, stromal-derived CXCL12 itself can stimulate the survival and growth of neoplastic cells in a paracrine fashion. Third, CXCL12 can promote tumor angiogenesis by attracting endothelial cells to the tumor microenvironment (5).

MATERIALS AND METHODS

Subject

Sixty Iraqi patients with bladder cancer were enrolled in this study. Thirty-nine male and 21 female, their aged were ranged between (35-82) years. They have been classified according to their stage and grade basic on WHO classification criteria. Also, 40 age-matched apparently healthy volunteers were included in this study.

Estimation of SDF-1 level

The level of SDF-1were determined by using Enzyme-linked immunosorbent assay (ELISA) by the kit from the GenAsia company, Philippines.

Genetic Analysis

Blood samples were collected from subjects involved in this study. Five ml of blood have been taken from them, and then was divided into to 2 ml put in EDTA tube (for DNA isolation / PCR / Sequencing). The extraction of DNA from samples was done by using gSYNC Genomic DNA Extraction Kit, Geneaid Company, Korea. This primer was designed by using a Primer-Blast feature of NCBI database to amplify an upstream sequence in the SDF-1 gene which included SH_rs1801157. The forward primer was 5´_CCA GCT CTG AAA CCA GTG TTA_3´ and the reverse 5´_CAG TCG TGG ACA CAC ATG AT_3´. PCR was carried out for all primers groups in a total volume of 25 µl. GoTaq Green Master mix in 12.5 µl, Forward and Reverse Primer as 1 µl for each one, Nuclease-Free Water 7.5 µl and 3.5 µl DNA. The reaction

components were mixed together in PCR tubes and then placed in PCR device. The PCR cycling conditions starting by an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation for 30 sec. at 95 °C, annealing for 1 min. at 60° C and the final extension for 1 cycle at 72° C for 9 min.

Statistical analysis

Serum level of cytokines was statically analyzed using SPSS program version 3. Their data were shown as a mean ± Standard error (S.E.) and the differences between means were assessed by ANOVA (Analysis of Variance) followed by LSD (Least Significant Difference) or Duncan test. Data of genotypes and alleles of the chemokine were recorded by using simple statistical parameters such as frequencies and significant difference between their distribution in BC patients and healthy subjects were assessed by two-tailed Fisher's exact probability (p). In addition, the Odd ratio (RR), an etiological fraction (EF) and preventive fraction (PF) were also calculated to define the association between genotypes with the disease. Allele frequencies were estimated by direct gene counting method and H-W calculator for two alleles was used to estimate the significant departure from Hardy-Weinberg (H-W) equilibrium using H-W calculator for two alleles, which is available free online at http://www.had2know.com/academics/hardy-Weinberg-equilibrium-calculator-2alleles.html. These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package (inducing the program and their manuals) is available free online at http:// www.brixtonhealth.com.

RESULTS AND DISCUSSION

The SDF-1 level and invasion

The SDF-1 level in figure (1) shows in results in healthy controls were significantly (2.093±0.05 ng/ml) lower than urinary bladder patients. The level of SDF-1 in Muscle Invasive Bladder Cancer (MIBC) was (3.727± 2.30 ng/ml) then followed by non-muscle invasive bladder cancer (NMIBC) (2.453 ± 0.47 ng/ml) which also have a significant difference between them.

The result revealed a significant increase in the level of SDF-1 in invasive bladder cancer. there is known from several kinds of studies that the modes of action are implicated in tumor pathogenesis; that promotes malignancy and cancer developments, participate in cancer metastasis, enhance tumor angiogenesis mediated cancer cell adhesion (6).

The CXCR4 is a typical G-protein-coupled receptor, and the combination of SDF-1 and CXCR4 can induce intracellular actin polymerization and cellular pseudopod formation, enhance cell movement ability and promote the cells to break through the limit of basement membrane and invasively grow(7).

The SDF-1 level and UBC grade

The results showed the highest level of SDF-1 were significantly higher in high grade of urothelial bladder cancer patients as (3.88±2.50 ng/ml) and low-grade patients as (2.57± 0.53 ng/ml). While the healthy control revealed a significant difference from the UBC patients as (2.09±0.05 ng/ml).

Result agreed with (8) who reported that CXCR4 is strongly expressed in high grade and Muscle-Invasive Bladder Cancer, although low-grade Non–Muscle Invasive Bladder Cancer which had a low level of CXCR. Because that normal urinary bladder expresses only decrepit CXCR4, the CXCR4 shows a low level of expression profiles mentioned that there is a potential of a new molecular target for the detection of the high-grade level of non-muscle invasive bladder cancer. As shown in figure (2).

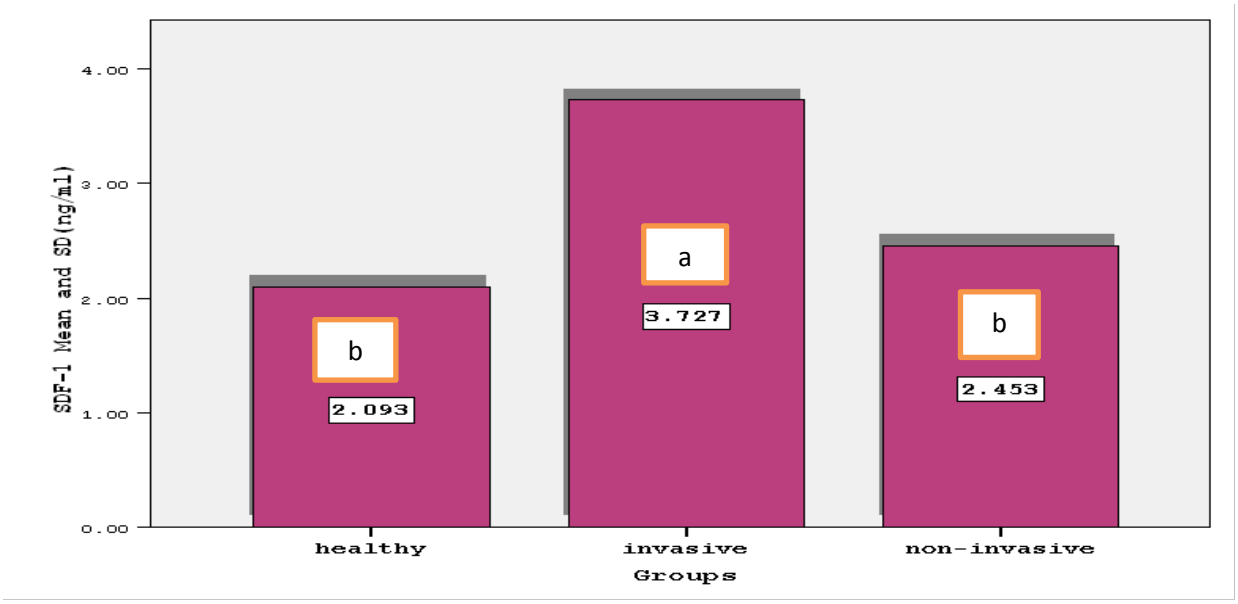

Figure (1): classification of SDF-1 level in urinary bladder cancer and healthy controls according to invasion and non-invasion types of cancer

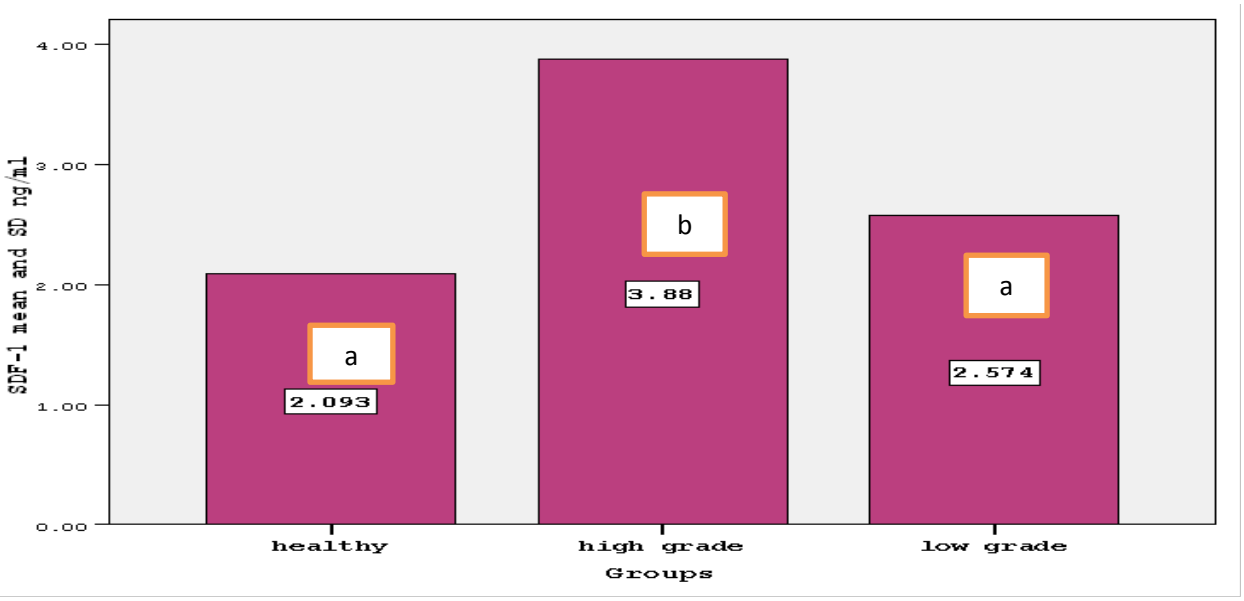

Figure (2): Serum SDF-1 level and tumor grade in urinary bladder cancer patients and healthy controls.

SDF-1 level and urinary bladder carcinoma stages

The serum SDF-1 level results that revealed in figure (3) showed a significantly higher level was reported in a T4 stage (6.396 ng/ml) which consider the most aggressive stage of bladder cancer, following by a T3 stage (4.190 ± 1.58 ng/ml) that shows a significantly difference results between them. Then, T2 (2.834 ±1.40 ng/ml) stage with a significant difference with T3 and T4. Then a T1 (2.545 ±1.03 ng/ml) stage also shows significant differences with stage T3 and T4 but no significant differences with T2. The healthy control had the no level (2.093±0.83ng /ml).

The interaction between SDF-1 and its receptors gives one of the importance about its function, which is regulating the tumor metastasis. CXCR4 might be playing a role to facilitate tumor dispersion at each of the main steps of metastasis, including the attachment of cancer cells to endothelium, extravasation from blood vessels, angiogenesis, metastatic colonization, and the escape from the host's response through the activation of certain pathways, and proliferation.

Many studies demonstrated the present evidence, that chemokines, such as SDF-1 facilitate communication between nonneoplastic cells and tumor cell in the tumor microenvironment, allows the infiltration, and the tumor-associated macrophages activation and neutrophils in the stromal (9)**.**

The sequencing results showed the intronic upstream (3'UTR) SNP (rs1801157) in SDF-1. Which was presented with three genotypes (GA, AA, and GG). The highest frequency was represented in the GG genotype which was found in 22 with frequency (36.6%) of UBC, followed by GA genotype found in 20 with frequency (33.3%) of UBC and AA genotype found in 18 (30%) of UBC respectively. Results of the healthy control showed GA genotype was found 22 (55%) followed by GG found in 10 (25%) and (20%) of the 8 healthy subjects showed as 8 genotypes. This result shows no significant difference was recorded between the three groups of the expected and observed frequencies (a good agreement with Hardy-Weinberg equilibrium) table (1).

The GG genotype frequency was increased in patients (36.7%) than in control (25%) and the odd ratio for such a positive association was 1.74 but with no significant differences (P= 0.28) and that may due to the low samples size. The G allele frequency was approximately the same in patients and in control. In contrast, decreased frequencies were observed with AA genotype and A allele in patients than in control. The odd ratio for such a positive association was (1.71) with no significant differences. The AG genotype frequency was lower in patients (33.3%) than in control (55.5%) and the odd ratio of this negative association was (0.41) with no significant differences Table (2).

SDF-1 chemokine level and (rs1801157) SNP.

The results in the figure (4) showed that serum SDF-1 AG genotype had a significant higher level of SDF-1 (3.584±0.59 ng/ml) followed by the GG genotype (2.763±0.66 ng/ml) and AA with the lowest level (2.643±0.71 ng/ml) while the healthy control revealed that AA genotype with high level of SDF-1 (2.36 ng/ml) followed by the GG genotype (2.16±0.84 ng/ml) and the GA genotype with the lowest level (2.099±0.66 ng/ml).

The *SDF1* gene occurs on chromosome 10q11.1. and protein has been suspected to be associated with an increased risk of various types of cancers, including lung cancer, breast cancer, bladder cancer, and acute leukemia(10). It suggested that *SDF1*-3′A polymorphism is identified as a G to A mutation at the position 801 in the 3′-UTR region in *SDF-1 β* transcriptional splice variant and *SDF1*-3′A polymorphism has been demonstrated to be associated with increased risk of multiple kinds of cancer, probably because its ability to increase the expression of SDF1 protein (11).

there was no significant difference about the distribution of GG, AG, and AA genotypes of SDF-1-(801) in patients with transitional cell carcinoma of the bladder and healthy people (12).

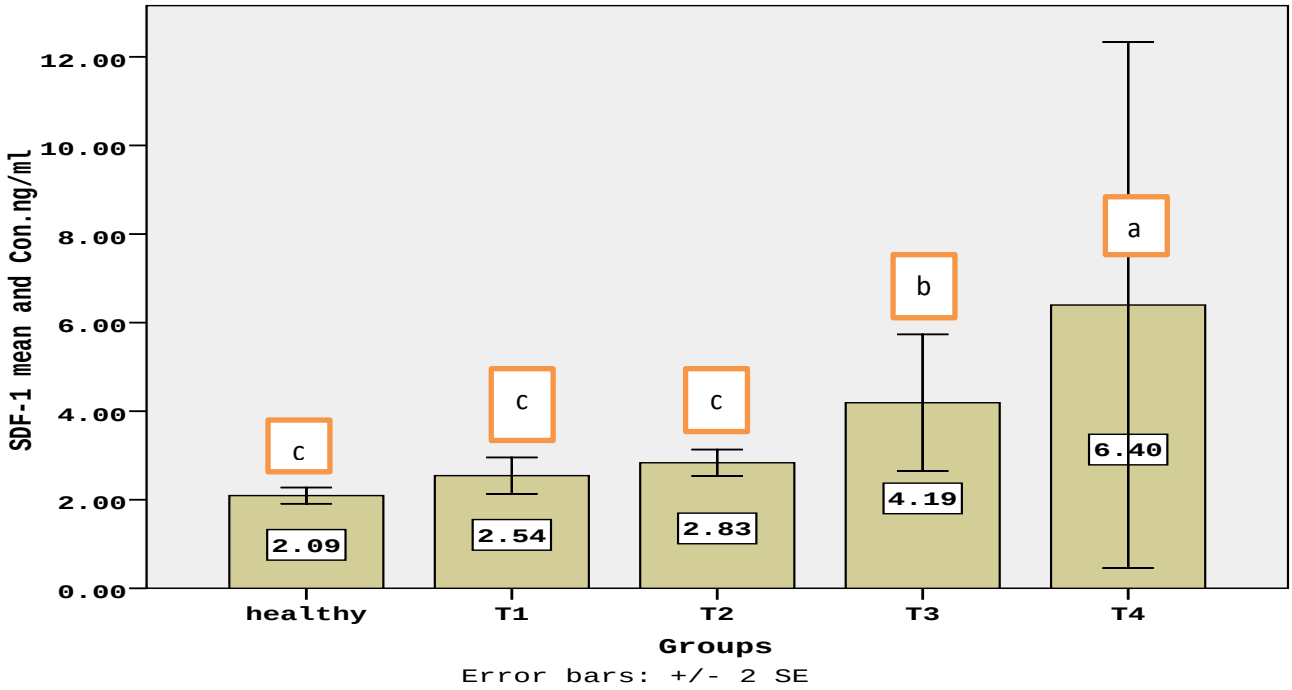

Figure (3): Serum SDF-1 level with cancer stages in urinary bladder carcinoma and controls individuals. SDF-1 gene (rs1801157) SNP

Table (1): Hardy-Wienberg equilibrium of SDF-1 rs1801157), Observed numbers and percentage frequencies in the Urinary Bladder Carcinoma and control.

Group			Genotype			H-W (p ≤)
			GG	GA	AA	
UBC	Observed	No.	22	20	18	N.S.
		%	36.6	33.3	30.0	
	Expected	No.	17.0	29.9	13.1	
		%	28.3	49.8	21.8	
Healthy	Observed	No.	10	22	8	N.S.
		%	25.0	55.0	20.0	
	Expected	No.	11.0	20	9.0	
		%	27.6	50.0	22.6	

Table (2): Statistical analysis of the association between SDF-1 rs1801157 genotype or allele in Urinary Bladder Carcinoma and control.

SDF-1 rs rs1801157 Genotype or Allele	Patient		Control		Statistical Evolution			
	No.	%	No.	%	Odd. Ratio	etiological or preventive fraction	Fisher's Exact Probability	95% Confidence Interval
GG	22	36.7	10	25.0	1.74	0.15	0.28	0.72 - 4.18
AG	20	33.3	22	55.5	0.41	0.32	0.031.	0.18 - 0.92
AA	18	30.0	8	20.0	1.71	0.12	0.35	0.67 – 4.39
G	64	53.3	42	52.5	1.03	0.02	1.00	0.59 - 1.82
A	56	46.7	38	47.5	0.97	0.02	1.00	0.55 -1.70

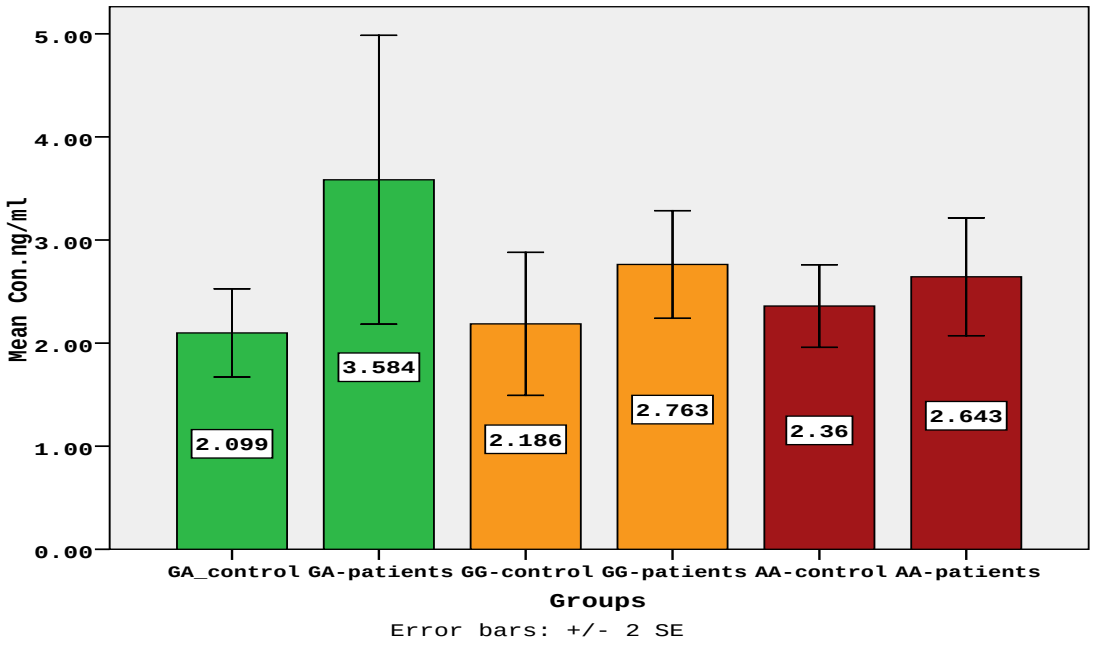

Figure (4): Serum SDF-1 level in Urothelial Bladder Carcinoma patients and healthy subjects and genotype at SNP (rs1801157).

CONCLUSION:

The SDF-1 level and genotypes results at (rs1801157) SNP showed that UBC patients with AG genotype have a significantly high level of SDF-1 followed by the GG genotype. While in healthy the GG showed the higher level followed by AA genotype and the AG showed the lowest level.

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