Archives of Razi Institute, Vol. 77, No. 3 (2022) 1059-1065

DOI: 10.22092/ARI.2022.357801.2098

Original Article

A Prognostic Impact of Interleukin 17 (IL-17) as an Immune-Marker in Patients with Bladder Cancer

Mousa, F. A[1], Jasim, H. A[1 *], Shakir, F[2]

1. Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq
2. Department of Surgery, College of Medicine, University of Basrah, Basrah, Iraq

Received 12 February 2022; Accepted 6 March 2022
Corresponding Author: Hanadi.jasim@uobasrah.edu.iq

Abstract

Urinary bladder cancer is a worldwide health issue and the ninth most prevalent cancer across the globe, accounting for almost two-thirds of all urinary malignancies. Interleukin 17 (IL17) is a pro-inflammatory cytokine with pivotal modulatory effects on antitumor immune responses and has been reported to play a prominent role in the occurrence and development of bladder cancer. The present study aimed to measure the quantitative serum and urine levels of IL-17 in patients with bladder cancer. Blood and urine samples were obtained from 50 diagnosed bladder cancers and 96 healthy people as a control group. The serum and urine level of IL-17 was evaluated using an enzyme-linked immunosorbent assay. It has been revealed that the level of IL-17 was higher in all patients, as compared to that in controls. These results indicated that this interleukin is an indicator to predict the progression or recurrence of the disease.

Keywords: Immune system, Carcinoma, Invasive cancer

1. Introduction

Urinary bladder cancer is a worldwide health issue (1), and the ninth most prevalent cancer across the globe, accounting for almost two-thirds of all urinary malignancies (1-3). Bladder cancer incidence in males is almost four times higher than that in females. In the patients who suffer from bladder cancer, the incidence of death dramatically increases with age, with roughly two-thirds of cases occurring in those aged 65 and over (3). There are many types of bladder cancer as follows: Type 1. Squamous cell carcinoma in which the squamous cells develop as a result of chronic irritation of the bladder, long and thin transitional cells gradually turned into squamous cells, and over time, these cells become cancerous, particularly with exposure to irritants or carcinogens. Squamous cell carcinoma accounts for about 4% of all bladder cancers (4-7). Type 2: In transitional cell carcinoma which is the most common type of bladder cancer (about 90%), cells from transitional cell carcinoma look like urothelial cells that line the bladder from the inside, and important factors influence the outlook for transitional cell carcinoma of cancer and whether or not it is invasive (8).

Bladder cancers are often classified based on how far they have spread into the wall of the bladder(1, 2) into non-muscle-invasive bladder cancer (Superficial) in which according to the Tumor, Node, Metastasis (TNM) classification system, papillary tumors are limited to the mucosa and entering the lamina propria are classified as stage Ta and T1, respectively. Carcinoma in situ (CIS) refers to flat high-grade tumors that are limited to the mucosa (Tis). Since these tumors can be treated by transurethral resection of the bladder

(TURB), eventually in conjunction with intravesical implants, they are classified as non-muscle-invasive bladder cancer (NMIBC) for therapeutic purposes (9). The other type is Muscle Invasive Bladder Cancer (deep cancer) in which a tumor is penetrating the muscular layer of the bladder wall (T2–T4), and it is found in around 25% of individuals with bladder cancer.

Despite rigorous local and systemic therapy, MIBC is linked to a high risk of recurrence and a poor overall prognosis. The 5-year death rate of individuals with MIBC remains about 50%-70% even after radical cystectomy (5). Due to the high risk of systemic failure among MIBC patients, a new multidisciplinary care paradigm has emerged, with systemic therapy playing an increasingly prominent role. It is hoped that the integration of surgery, medical oncology, and radiation oncology may enhance clinical outcomes for patients with MIBC (10).

Bladder cancers are also graded based on the appearance of the cancer cells under a microscope. Low-grade tumors have a similar appearance to normal bladder tissue. Well-differentiated cancers are another name for them, and the prognosis of these tumors is typically favorable. On the other hand, high-grade tumors have a less marked resemblance to normal tissues. These malignancies are also known as undifferentiated or weakly differentiated cancers. The high-grade malignancies are more likely to spread outside the bladder by growing into the bladder wall (1).

Interleukin (IL) 17 is produced by T-helper (Th) 17 with a vigorous effect on cells of the immune system, playing important roles in the pathogenesis of immune-mediated diseases, including autoimmune disorders and cancers (11). The IL-17 is a pro-inflammatory cytokine with pivotal modulatory effects on antitumor immune responses (5). Th17 as a subtype of CD4+ cells releases the cytokine IL-17 which plays a key role in the innate and adaptive immune systems. The two most significant subtypes of the IL-17 family, IL-17A and IL-17F, act by producing a variety of pro-inflammatory mediators, such as chemokines, metalloproteinase, and cytokines (12).

Th17 cells have been found to be predominant in the tumor tissues of bladder cancer patients, and an imbalance between Th-17 and Treg cells has been linked to the genesis or progression of the disease. The IL-17 is a pro-inflammatory cytokine that is thought to be one of the most effective anticancer cytokines. Several investigations, nonetheless, have indicated that the tumor promoter activity of this cytokine plays a particularly important role in the occurrence and development of bladder cancer (5). Some studies have demonstrated that IL-17 plays an intricate role in the pathophysiology of cancer, from tumorigenesis, proliferation, angiogenesis, and metastasis to adapting the tumor in its ability to confer upon itself both immune and chemotherapy resistance (13).

In light of the aforementioned issues, the present study aimed to measure the quantitative serum and urine levels of IL-17 in patients with bladder cancer.

2. Materials and Methods

2.1. Study Group

This case-control study was conducted on 50 patients with Bladder Cancer (BC), including 27 cases with non-muscle-invasive urinary bladder cancer and 23 subjects with muscle-invasive urinary bladder cancer (8 new cases and 42 recurrent cases of urinary bladder cancer). The participants were within the age range of 30-90 years (mean age of 65.40). This study was conducted in Al-Basra General Hospital and Almawanee Hospital from March-2021 to October-2021. The majority of patients were diagnosed by a clinician. Moreover, 96 apparently healthy individuals were included in this study as a control group with a mean age of 64.11 years.

All enrolled patients presented with bladder tumor, non-muscle-invasive, and muscle-invasive (first presentation); moreover, some patients had recurrent bladder tumors. It is worth noting that patients with hematuria, acute urinary tract infection, and urinary

stones were excluded from the study.

2.2. Measuring Serum and Urine Level of IL-17 by Enzyme-Linked Immunosorbent Assay

A total of 5 mL of peripheral blood samples were collected in vacuum gel tubes, and urine samples (50 mL) were also collected from patients and control group in sterile cups and centrifuged to obtain serum and precipitated urine and then stored at - 35°C for use in a serological study by Enzyme-Linked Immunosorbent Assay (ELISA). Serum and urine IL17 concentrations were estimated by specific commercial kits, Sandwich Enzyme-Linked Immunosorbent Assay (IL-17 ELISA Kit provided by Bio Sources, USA). The assays were measured using an ELISA reader at 450 nm.

2.3. Statistical Analysis

The data were analyzed in SPSS software (version 24). Qualitative data are presented as frequencies and percentages, while quantitative data are displayed as mean± standard deviation, median, as well as minimum and maximum values. To test for the normality of quantitative data distribution, Kolmogorov Smirnov and Shapiro Wilk tests were used. To investigate the statistical significance in any observed association of qualitative variables, the chi-squared test was used. Moreover, the Mann-Whitney U test, Wilcoxon signed-rank test, and Kruskal Wallis tests were used to assess the presence of significant statistical differences between/among quantitative variables. Spearman's nonparametric correlation test was used to decide on the presence of statistically significant correlations.

3. Results

A case-control study of bladder cancer patients was conducted according to the minimum sample size equation that depends on the percentage of the disease, and 50 bladder cancer patients within the age range of 30- 90 years participated in this study. Moreover, 96 individuals were regarded as the control group after being examined and ensured that they are free of any urinary tract problems or other clinical problems. According to the distribution of the study groups, there were no statistically significant differences between patients and controls when classified according to gender, age, and smoking status (Table 1).

Table 1. Distribution of the patients and controls according to sociodemographic characteristics

	Category		Total	*P*-value
	Patient	Control		
Male	39 78.0%	64 66.7%	103 70.5%	*P*= 0.154*
Female	11 22.0%	32 33.3%	43 29.5%	
Age (year) Mean± SD **Median** **Min.- Max.**	65.40±11.68 66.50 40-85	64.11±16.34 65.50 39-92		*P*= 0.66**
Smoker	32 64.0%	52 54.2%	84 57.5%	*P*= 0.254*
Non Smoker	18 36.0%	44 45.8%	62 42.5%	
Total	50 100.0%	96 100.0%	146 100.0%	

* chi-squared test, ** Mann-Whitney U Test

The differences in the levels of interleukin-17 between patients and controls were found to be statistically significantly higher in the patient group (Table 2). In addition, when the differences between serum and urine samples were tested, they were found to be significantly higher in serum.

Interleukin-17 serum and urine levels according to different age groups in patients and control groups did not demonstrate any statistically significant difference (Table 3).

Regarding differences in the levels of IL-17 in serum and urine samples according to the stage of the disease, table 4 illustrates that serum levels were significantly higher in patients with muscle invasiveness.

Regarding the positive history of smoking, no significant differences could be elicited in the levels of IL-17 in serum and urine samples (Table 5).

With respect to the disease diagnosis, whether new or recurrent, no significant difference was recorded (Table 6).

Table 2. Differences in the levels of interleukin-17 between patients and controls and its levels between serum and urine

Category		Serum IL-17	Urine IL-17
Patient	N	50	50
	Mean± SD	48.26±25.49	10.23±5.77
	Median	48.60	8.78
	Min.- Max.	11.20-105.00	3.33-29.90
Control	N	96	96
	Mean± SD	0.62±0.41	0.72±0.44
	Median	0.58	0.63
	Min.- Max.	0.10-2.45	0.10-2.45
Sig.*		0.0001	0.0001
Sig.**		0.0001	

* Mann-Whitney U Test, ** Wilcoxon Signed Ranks Test

Table 3. Differences in interleukin-17 serum and urine levels according to different age groups in patients and controls

Category	Age groups		Serum IL-17	Urine IL-17
Patients	Younger than 60 years	N	13	13
		Mean± SD	39.03±25.68	10.74±5.78
		Median	40.20	8.93
		Min.-Max.	15.20-105.00	4.12-25.60
	From 60 to 80 years	N	32	32
		Mean± SD	52.73±24.74	9.92±5.69
		Median	53.25	8.33
		Min.-Max.	11.20-105.00	3.33-29.90
	Older than 80 years	N	5	5
		Mean± SD	43.60±27.87	10.93±7.41
		Median	62.10	9.30
		Min.-Max.	12.30-65.00	4.30-23.30
Sig.*			0.125	0.783
Controls	Younger than 60 years	N	39	39
		Mean± SD	0.72±0.49	0.69±0.35
		Median	0.60	0.63
		Min.-Max.	0.14-2.45	0.23-1.60
	From 60 to 80 years	N	38	38
		Mean± SD	0.56±0.31	0.75±0.45
		Median	0.57	0.65
		Min.-Max.	0.10-1.50	0.10-1.80
	Older than 80 years	N	19	19
		Mean± SD	0.55±0.35	0.74±0.56
		Median	0.42	0.51
		Min.-Max.	0.15-1.70	0.18-2.45
Sig.*			0.340	0.800

* Kruskal Wallis Test

Table 4. Differences in interleukin-17 serum and urine levels according to the stage of the disease

Stage		Serum IL-17	Urine IL-17
NMIBC	N	27	27
	Mean± SD	42.55±25.42	9.98±5.95
	Median	44.20	7.98
	Min.- Max.	12.30-99.00	3.33-29.90
MIBC	N	23	23
	Mean± SD	54.95±24.44	10.52±5.67
	Median	55.20	8.78
	Min.- Max.	11.20-105.00	5.77-24.20
	Sig.*	0.040	0.961

* Mann-Whitney U Test

Table 5. Differences in interleukin-17 serum and urine levels according to smoking history

Smoker		Serum IL-17	Urine IL-17
Yes	N	32	32
	Mean± SD	48.89±24.44	10.78±6.73
	Median	53.25	8.38
	Min.- Max.	11.20-99.00	4.12-29.90
No	N	18	18
	Mean± SD	47.12±27.97	9.25±3.46
	Median	44.20	9.01
	Min.- Max.	13.90-105.00	3.33-16.40
	Sig.*	0.518	0.887

* Mann-Whitney U Test

Table 6. Differences in interleukin-17 serum and urine levels according to the type of occurrence

Diagnosis		Serum IL-17	Urine IL-17
Recurrent	N	42	42
	Mean± SD	47.96±22.91	10.73±6.11
	Median	50.20	8.83

4. Discussion

The induction of the secretion of cytokines and chemokine has been proposed by the activity of IL-17 as a proinflammatory key of many cell types among which myeloid cells and mesenchymal cells can recruit the cells for inflammation monocytes (6, 14). In addition, substantial evidence has suggested that IL-17 markedly causes angiogenesis and tumor growth, indicating the important role of IL-17 in tumor promotion (15).

The non-significant differences in gender, age, and smoking between patients and controls made it possible to compare differences between the two groups. Regarding the difference in serum and urine IL-17 levels between the two groups, the levels were found to be significantly higher in patients. This agrees with the findings of a study by Fattahi, Karimi (7) who documented higher IL-17 in bladder cancer tissues than in non-bladder cancer tissues in the control group. Moreover, the levels were higher in serum than in the urine; nonetheless, there was a significant positive correlation between the two levels (r= 59.8%), which is expected as the increase in its serum level excreted in the urine. This is consistent with the results of a study by Baharlou (11) who investigated such an association with other cancers and since IL 17 is an inflammatory cytokine, it increases markedly in the cancer process.

In addition, its serum levels were significantly higher with muscle cancer invasiveness, which goes with this fact. Consequently, when its levels increase in serum, it increases in urine as well. These elevations do not vary with age and this study failed to diagnose any variation between different age groups. This is inconsistent with the findings of a study by De Angulo, Faris (16) who documented that an increase in age is associated with the elevation of pro-inflammatory cytokines, including IL-17. This is probably due to the fact that people in Western countries have a longer life expectancy, as compared to Iranian people.

Smoking did not show any association with a significant difference in the levels of interleukin 17. This finding differs from the results of a study by Huang, Wang (2) who demonstrated an increased expression of IL-17 in smokers. This can be ascribed to different habits in research populations since some people are addicted to alcohol in addition to smoking. The same finding was found when the recurrence was considered. This finding is different from the results of a study by Song, Yang (17) who found an association between IL-17 and other cancers. This may be attributed to the fact that interleukin-17 is a predictor of cancer.

As evidenced by the results of this research, it can be concluded that interleukin 17 can be considered a biomarker for the evaluation of some serum and urinary cytokine levels as non-invasive diagnostic tools in the prediction of bladder cancer. Serum and urine levels of cytokine increase in patients, as compared to those in controls; moreover, the levels were higher in serum than urine in patients. Furthermore, the serum level was significantly higher in muscle-invasive bladder cancer.

Authors' Contribution

Study concept and design: F. A. M.
Acquisition of data: F. A. M.
Analysis and interpretation of data: F. A. M.
Drafting of the manuscript: F. A. M.
Critical revision of the manuscript for important intellectual content: F. A. M.
Statistical analysis: H. A. J. and F. S.
Administrative, technical, and material support: F. A. M.

Ethics

The study was approved by the Ethics Committee of the College of Medicine, University of Basrah.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors' deepest appreciation goes to all the employees of the Department of Microbiology, College of Medicine, University of Basra, and the supervising professors. We also thank the staff of Basra Teaching Hospital and Al Mawanee Hospital, especially the laboratory and urology staff, for their generous support. Our special gratitude is extended to Assistant Professor Dr. Alaa Hussein Abed, Basha University College of Science and Technology, for his help in statistical analysis.

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