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# Interleukin-2 and Interleukin-10 Prognostic effect in Patients with Bladder Cancer

**Fatima A. Mousa[a], Hanadi Abdulqader Jasim[b], Firas Shakir Attar[c]**

*a ,c: Department of Microbiology, College of Medicine, University of Basrah, Iraq*

*b: Department of Surgery, College of Medicine, University of Basrah, Iraq*

*Corresponding author: Hanadi.jasim@uobasrah.edu.iq*

## Abstract

Background: Bladder cancer is one of the most common tumors of the urinary tract and one of the main causes of cancer-related death in males. It is the ninth most prevalent cancer globally. The microenvironment of a bladder tumor has been indicated to be rich in growth factors/inflammatory cytokines that can induce tumor growth/progression and also suppress the immune system.

Methods: This is a case-control study in which patients with Bladder Cancer were enrolled. Blood and urine samples were obtained from 50 diagnosed patients and 96 healthy people as a control group. Interleukin levels were evaluated. In this study, quantitative levels of serum and urine interleukin2 and interleukin 10 in bladder cancer patients were evaluated by using ELISA test.

Results: It has been found that the level of these cytokines was higher in all patients as compared with their level at the control group. There were highly significant differences in the levels of interleukin-2 between patients and controls ($P$=0.0001), which is higher in the patient group. Additionally, the serum IL-2, (133.5 > 0.48) was found to be significantly higher than urine IL-2, (113.5 > 0.44), ($P$= 0.021). While there was no significant difference in the levels between serum and urine IL -10 (P= 0.825) in patients and control. These results indicated that interleukin 2 as an indicator to predict the progression or recurrence of the disease.

Conclusion: In Conclusion, Interleukins probably may play a role in predicting the progression of bladder cancer or the development of advanced disease. Serum and urine levels of cytokine increased in the patient's comparison with the control, also the levels were higher in serum than the urine in patients

**Keywords**: Bladder cancer, urinary tract, growth factors, healthy people, Basra, Iraq

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## Introduction

Bladder cancer is one of the most common tumors of the urinary tract and one of the main causes of cancer-related death in males (Jemal et al, 2007).

Growth factors and inflammatory cytokines have been found to be abundant in the microenvironment of bladder tumors, which can promote tumor development and progression while inhibiting the immune system. Activation of cytokine-related pathways, on the other hand, has been linked to a decrease in cancer progression, implying that cytokines could be utilized as therapeutic targets (Metts *et al*, 2000).

The tumor, node, and metastases (TNM) system is used to characterize the pathological stage of bladder cancer (American Joint Committee on Cancer, 2010). Urologists, radiotherapists, and oncologists employ stage grouping once all of the clinical data has been gathered (Dougal, 2008).

Only about 1% of bladder cancers are adenocarcinomas. These cancer cells have a lot in common with gland-forming cells of colon cancers. Nearly all adenocarcinomas of the bladder are invasive (American Cancer Society, 2019). On the other hand, squamous cells develop as a result of chronic irritation of the bladder, in which the thin and long transitional cells gradually turn into squamous cells, and over time these cells become cancerous especially with exposure to irritants or carcinogens. Squamous cell carcinoma accounts for about 4% of all bladder cancers. (Rausch et al., 2012)

The cytokine messenger protein interleukin-2 (IL-2) stimulates components of the immune system. It is a member of the interleukins family, which was first identified in 1976. Despite the structural similarities across members of this family, IL-2 has made a significant contribution to the field of immunotherapy in general and cancer therapy in particular. IL-2 is particularly significant since it controls both inflammatory and immunological responses by increasing blood cell development in the immune system. Many investigations have identified IL-2 as the lymphocytotropic cytokine responsible for triggering the proliferation of helper T-lymphocytes (CD4 T-cells)(Shaker and Younes, 2009).

In bladder cancer, IL-10 acts as a Th1 inhibitory cytokine. Patients with bladder cancer have a Th2 dominant immune response with a poor Th1 immune response, according to studies. Increased amounts of IL-10 and other Th2 cytokines (e.g., IL-4, IL-5, and IL-6) have been seen in the serum of bladder cancer patients, as well as lower levels of Th1 cytokines (e.g., IFN- and IL-2) (Agarwal et al., 2010; Satyam et al., 2011).

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The aim of this study was to evaluate serum and urinary levels of IL-2 and IL-10 as a tool in prediction of bladder cancer.

## Materials and Methods

### Study group

This is a case-control study in which fifty patients with Bladder Cancer were enrolled (27 non-muscle -invasive urinary bladder cancer and 23 muscle –invasive urinary bladder cancer); (8 new cases and 42 recurrent case urinary bladder cancer), aged from (30-90) year old with the mean age of (65.40). This study was conducted through the period from March-2021 till October-2021 from Al-Basra General Hospital and Al-mawanee Hospital. Most those patients are diagnosed by clinician. Also (96) apparently healthy persons were involved in this study as a control group with mean age of (64.11) year-old.

All enrolled patients presented with bladder tumor, non-muscle invasive and muscle invasive (first presentation) and patients with recurrent bladder tumors. The excluded patients included those had hematuria, acute urinary tract infection and urinary stones.

### Measuring serum and urine level of IL-2 and IL-10 by Enzyme Linked Immunosorbent Assay

Five milliliters (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 ELISA. Serum and urine IL10 and IL-2 concentrations were estimated by specific commercial kits, Sandwich Enzyme-Linked Immunosorbent Assay (IL-10 and IL-2 ELISA Kit provided by Bio Sources, USA). Assays were measured using an ELISA reader at 450 nm.

### Statistical analysis:

For the purpose of statistical analysis of data, SPSS software, version 24, was used. Qualitative data are shown as frequencies and percentages, while quantitative data are presented as mean± standard deviation, median, and 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, $Chi^2$ was used; and to investigate for the presence of significant statistical differences between/ among quantitative variables Mann-Whitney U, Wilcoxon Signed Ranks, and Kruskal Wallis tests were used. Spearman's

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nonparametric correlation test was used to decide about the presence of statistically significant correlations.

**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 the total number of bladder cancer patients participating in this study was (50) individuals with age ranged between (30- 90) years and (96) individuals are considered a control group after being examined and ensured that they are free of any urinary tract problems or any 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 sex, according to age, or according to smoking (Table 1).

**Table (1): Distribution of the patients and control according to socio-demographic characteristics**

| | Category | | Total | Sig. |
|---|---|---|---|---|
| | Patient | Control | | |
| **Sex** | | | | |
| **Male** | 39 | 64 | 103 | P= 0.154* |
| | 78.0% | 66.7% | 70.5% | |

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| | | | | |
|---|---|---|---|---|
| **Female** | 11 | 32 | 43 | |
| | 22.0% | 33.3% | 29.5% | |
| **Age (year)** | | | | |
| **Mean± SD** | 65.40± 11.68 | 64.11±16.34 | | |
| **Median** | 66.50 | 65.50 | | P= 0.66** |
| **Min.- Max.** | 40-85 | 39-92 | | |
| **Smoking** | | | | |
| **Yes** | 32 | 52 | 84 | |
| | 64.0% | 54.2% | 57.5% | P= 0.254* |
| **No** | 18 | 44 | 62 | |
| | 36.0% | 45.8% | 42.5% | |
| **Total** | 50 | 96 | 146 | |
| | 100.0% | 100.0% | 100.0% | |

* $Chi^2$Test, ** Mann-Whitney U Test

In Table (2) the differences in the levels of interleukin-2 between patients and control were found to be statistically significantly higher in the patient group. Also, when the differences in its levels between serum and urine were tested, they were found significantly higher in serum.

**Table (2): Differences in the levels of interleukin-2 between patients and controls and its levels between serum and urine**

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| Category | | S. IL-2 | U.IL-2 |
|---|---|---|---|
| Patient | N | 50 | 50 |
| | Mean± SD | 136.15±46.20 | 120.35±54.89 |
| | Median | 133.50 | 113.50 |
| | Min.- Max. | 56.41-257.00 | 37.00-254.0 |
| Control | N | 96 | 96 |
| | Mean± SD | 0.53±0.31 | 0.47±0.24 |
| | Median | 0.48 | 0.44 |
| | Min.- Max. | 0.12-1.50 | 0.11-1.10 |
| Sig.* | | 0.0001 | 0.0001 |
| Sig.** | | 0.021 | |

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

The differences in the levels of interleukin-10 between patients and controls were found to be statistically significantly higher in patients. However, no such significance was found in the differences in its levels between serum and urine (Table 3).

**Table (3): Differences in the levels of interleukin-10 between patients and controls and its levels between serum and urine**

| Category | S.IL-10 | U.IL-10 |
|---|---|---|

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| | | | |
|---|---|---|---|
| Patient | N | 50 | 50 |
| | Mean± SD | 32.49±20.66 | 38.23±16.88 |
| | Median | 24.85 | 31.20 |
| | Min.- Max. | 16.40-98.10 | 17.50-82.30 |
| Control | N | 96 | 96 |
| | Mean± SD | 3.73±2.61 | 2.57±2.22 |
| | Median | 3.17 | 2.20 |
| | Min.- Max. | 0.12-15.20 | 0.10-7.30 |
| Sig.* | | 0.0001 | 0.0001 |
| Sig.** | | 0.825 | |

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

**Table (4): Spearman's nonparametric correlations between the studied interleukins serum and urine levels**

| **Marker** | | |
|---|---|---|
| Serum IL-2 with Urine IL-2 | R | 0.660 |
| | Sig. | 0.0001 |
| Serum IL-10 with Urine IL-10 | R | 0.719 |
| | Sig. | 0.0001 |

In Table (5), it is noticed that there were statistically significantly higher serum and urine levels of interleukin-2 in patients with muscle invasiveness that in patients with no muscle invasiveness.

No any significant statistical difference in the levels of interleukin-10 was found, as shown in Table (6).

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**Table (5): Differences in interleukin-2 serum and urine levels according to the stage of the disease**

| Stage | | Serum IL-2 | Urine IL-2 |
|---|---|---|---|
| NMIBC | N | 27 | 27 |
| | Mean± SD | 118.34±36.86 | 98.27±48.49 |
| | Median | 123.00 | 88.00 |
| | Min.- Max. | 56.41-181.00 | 39.00-254.00 |
| MIBC | N | 23 | 23 |
| | Mean± SD | 157.06±47.99 | 146.26±51.28 |
| | Median | 149.00 | 154.00 |
| | Min.- Max. | 61.20-257.00 | 37.00-216.00 |
| | Sig.* | 0.003 | 0.001 |

* Mann-Whitney U Test

**Table (6): Differences in interleukin-10 serum and urine levels according to the stage of the disease**

| Stage | | Serum IL-10 | Urine IL-10 |
|---|---|---|---|
| NMIBC | N | 27 | 27 |
| | Mean± SD | 32.44±18.69 | 34.98±16.13 |
| | Median | 25.50 | 28.80 |
| | Min.- Max. | 16.80-84.90 | 17.50-81.20 |
| MIBC | N | 23 | 23 |
| | Mean± SD | 32.54±23.18 | 42.03±17.28 |

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| | | | |
|---|---|---|---|
| | Median | 22.50 | 39.80 |
| | Min.- Max. | 16.40-98.10 | 21.30-82.30 |
| | Sig.* | 0.763 | 0.188 |

* Mann-Whitney U Test

## Discussion

### Interleukin -2 and bladder cancer

Bladder transitional cell carcinoma is one of the solid tumors, that is mostly responsive to immunotherapy. The interleukin-2 (IL-2) production appears to be an important requirement for immunotherapy to be effective in the management of bladder cancer (Kausch et al., 2002). As IL-2 plays a crucial role in the immunity by functioning as an autocrine and paracrine mediator in the expansion and differentiation of lymphocytes as well as natural killer cells (NK) of the cytotoxic cell system (Gaffen and Liu, 2004). Furthermore, various other cytokines and factors are enhanced by the effect of IL-2, as the TNFa or IFN by different pathways to impact "the survival, effector function, and apoptosis" of immune system (Bachmann and Oxenius, 2007).

In the current results, there are high significant differences in the levels of interleukin-2 between patients and control ($P$=0.0001), which is higher in the patient group. Additionally, the serum IL-2, (133.5 > 0.48) found to be significantly higher than urine IL-2, (113.5 > 0.44), ($P$= 0.021) (Table2). Although, spearman's non-parametric correlations showed a positive association between two arms ($R$= 0.66; $P$= 0.0001) (Table 4). Several studies support our findings regarding IL-2 such as (Jiang et al., 2016; Babjuk et al., 2019).

In relation to stage cancer invasiveness, we observed a significantly higher serum and urine levels of IL-2 in advanced invasive muscle tumor than early non muscle invasive stage (P= 0.003; 0.001), respectively (Table 5). We suggested that the concentration in IL-2 is more in advanced bladder cancer than in the early stage as a results of cancer cells proliferation and disease progression, which is more obviously in urine (154 > 88) than in serum (149 > 123).

### Interleukin-10 and bladder cancer

Interleukin 10 is a potent anti-inflammatory cytokine that plays an essential role in preventing inflammatory and autoimmune pathologies (Sabat et al., 2010). Deficiency

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of IL-10 can influence inflammatory response to microbial challenge and lead to development of a number of autoimmune diseases (O'Garra et al., 2008). In relation to IL-10 level, the differences in the levels of IL-10 between the two arms are statistically significant which were higher in the patients (P= 0.0001). However, there was no significant difference in the levels between serum and urine IL -10 (P= 0.825) (Table 3), yet, there is a positive correlation between two arms by spearman's non-parametric (R= 0.719; P= 0.0001) (Table 4). Our results about IL-10 were similar to that observed by Ruffell et al., (2014) and by Loser et al., (2007), who reported an increasing over expression of IL-10 in cancer cell as compared to healthy cells in several animal models. Thus, IL-10 may help in some types of cancer treatment as an immunotherapy or target therapy by potentiating the activity of antitumor CD8 T cells (Autio and Oft, 2019; Ouyang and O'Garra, 2019; Wang et al., 2019).

The IL-10 levels have no correlation in both early (non-invasive) and advanced (invasive) stages of bladder cancer whether in serum or in urine (*P*= 0.763, 0.188), respectively (Table 6). We suggest that IL-10 have no role in the stage or invasiveness of bladder cancer, but this suggestion cannot be proven because there is no statistical significant difference in our results

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**Ethical consideration**

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

**Conflicts of Interest**

The authors declare that they have no competing interests.

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