

## **Some Inflammatory Cytokine Profiling in Bladder Cancer Patients with and without Urinary Tract Infections**

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### **Summary**

**Background:** Excessive inflammation in bladder cancer cases often leads to complications, and UTIs have been shown to strengthen existing bladder inflammation. IL-1 $\beta$  and TNF- $\alpha$  function as crucial during this process. The inflammatory mediators, pro-inflammatory cytokines, guide infection defense against UTIs and cancer susceptibility and progression.

**Aim of the study:** The first objective of this research was to assess and differentiate IL-1 $\beta$  and TNF- $\alpha$  concentrations in bladder cancer patients with recurring or without UTIs. This research examined how UTIs affect bladder cancer patients' inflammatory cytokines in their bloodstream while studying whether such infections correlate with cytokine measurements.

**Methods:** Researchers obtained 76 blood samples totaling 2ml from bladder cancer patients throughout the Oncology Center in Najaf Governorate from June 2024 until December 2024. This investigation included patient populations between the ages of 45 to 75 years. Researchers measured IL-1beta (IL-1 $\beta$ ) and tumor necrosis factor (TNF- $\alpha$ ) concentrations through bladder cancer samples obtained from 40 patients with UTIs versus samples obtained from 36 patients who did not develop UTIs. Blood samples were collected from healthy individuals to serve as a control group. **Results:** This study analyzed 76 blood samples from bladder cancer patients, 40 of whom also had a urinary tract infection (UTI), including 30 males and 10 females aged 50–75. The remaining 36 patients, all males aged 60–70, had bladder cancer without UTI. Additionally, 30 healthy individuals served as controls. Immunological analysis showed significantly elevated levels of TNF- $\alpha$  and IL-1 $\beta$  in bladder cancer patients with UTI compared to those without UTI and the control group. Notably, TNF- $\alpha$  levels were highest in the UTI group ( $1.7525 \pm 0.0693$ ) and progressively lower in non-UTI patients and controls. A similar pattern was seen for IL-1 $\beta$ , with UTI patients showing the highest levels ( $1.7217 \pm 0.0387$ ), followed by non-UTI patients ( $1.2705 \pm$

0.0557), and controls ( $0.7585 \pm 0.0458$ ). These differences were statistically significant ( $p = 0.0001$ ).

**Keywords:** Cytokine Profiling, Bladder Cancer, Urinary Tract Infections.

## **Introduction**

The most prevalent urinary bladder malignancy exists as a diverse disease that necessitates medical intervention between bladder surveillance and radical cystectomy treatment options. Urinary bladder cancer cases are classified through tumor grade and tumor stage analysis using the newly updated American Joint Committee on Cancer (AJCC) Staging Manual, 8th edition [1]. Urinary tract infection (UTI) represents a common health issue in patients who have bladder cancer, given that tumor presence might lead to urinary tract changes that promote infection [2]. The health danger from UTIs results in medical complications that affect millions of people annually [3]. Furthermore, most microbiological screenings are linked with cases of potential UTIs [4]. Research into the relationship between UTI and bladder cancer development needs evaluation of inflammatory reactions like  $\text{TNF}\alpha$  because UTI remains common among bladder cancer patients.  $\text{TNF}\alpha$  emerges as a pleiotropic proinflammatory cytokine that follows is produced by macrophages and neutrophils, fibroblasts, keratinocytes, NK

cells, T and B cells, and tumor cells.  $\text{TNF}\alpha$  protects the body from tumors and infections, but acute and chronic inflammatory responses operate as the active defenses of the host [5]. The inflammatory protein  $\text{IL-1}\beta$  functions to advance bladder cancer development and regulate UTI responses [6]. UTI-related inflammation benefits from  $\text{IL-1}\beta$  by activating immune cell recruitment and increasing local inflammation, resulting in tissue damage and additional health complications [7].

## **Materials and Methods**

### **Samples Collection**

The researchers collected 76 blood samples from bladder cancer patients at Najaf Governorate Oncology Center from June 2024 to December 2024. The research examines two patient groups: the first included 40 bladder cancer patients with UTI history, and the second included 36 bladder cancer patients without UTI history. Moreover, 30 blood samples were collected from healthy people as the control group.

## Immunological Profile

(2 ml) of blood collected, then centrifugation was performed to obtain serum that remained in storage at  $-20^{\circ}\text{C}$  before testing. The key inflammatory cytokines IL- $1\beta$  and tumor necrosis factor (TNF- $\alpha$ ) were detected by employing ELISA kits purchased from Elabscience (USA) through manufacturer-defined procedures.

## Statistical Analysis

SPSS software allowed a one-way ANOVA test to evaluate the IL- $1\beta$  and TNF- $\alpha$  levels between bladder cancer patients with UTIs, those without UTIs, and healthy controls. Statistical evaluation between these groups produced significant results because the calculated p-value fell below 0.05.

## Results and Discussion

### Bladder cancer patients

This study included 76 (100%) clinical specimens (blood) obtained from patients diagnosed with bladder cancer. Among them, 40 (52.6%) patients had bladder cancer accompanied by UTI, divided into 30 (75%) males and 10 (25%) females.

These patients ranged in age from 50 to 75 years. The remaining 36 (47.4%) patients had bladder cancer without UTI, and all were males aged between 60 and 70 years. Additionally, 30 (100%) healthy individuals were included as a control group for comparison.

### Immunological Analysis of Interleukins

TNF- $\alpha$  levels ( $1.7525 \pm 0.0693$ ) in bladder cancer patients with UTI reached elevated levels compared to those without UTI ( $1.1102 \pm 0.0655$ ) and the control group ( $0.7976 \pm 0.2683$ ). There was also a significant difference between bladder cancer patients without UTI ( $1.1102 \pm 0.0655$ ) and the control group ( $0.7976 \pm 0.2683$ ). IL- $1\beta$  concentrations showed a similar distribution pattern to TNF- $\alpha$ , displaying elevated levels in UTI patients ( $1.7217 \pm 0.0387$ ) and then decreasing to lower levels in non-UTI patients ( $1.1102 \pm 0.0655$ ) and the control group ( $0.7585 \pm 0.0458$ ). There was also a significant difference between bladder cancer patients who did not have urinary tract infection ( $1.2705 \pm 0.0557$ ) compared to the control group ( $0.7585 \pm 0.0458$ ) at a p-value of 0.0001 (Table 1).

| Markers       | BC patients with UTI<br>Mean $\pm$ Sd | BC patients without UTI<br>Mean $\pm$ Sd | Control<br>Mean $\pm$ Sd | P-<br>Value |
|---------------|---------------------------------------|------------------------------------------|--------------------------|-------------|
| TNF- $\alpha$ | 1.7525 $\pm$ 0.0693                   | 1.1102 $\pm$ 0.0655                      | 0.7976 $\pm$ 0.2683      | 0.0001      |
| IL-1 $\beta$  | 1.7217 $\pm$ 0.0387                   | 1.2705 $\pm$ 0.0557                      | 0.7585 $\pm$ 0.0458      | 0.0001      |

## Discussion

According to (8), Patients' ages ranged from 29 to 90 years, with the highest frequency of UBC occurring in the age group of 60-79 years (62.9%), 58 males (82.9%), and 12 females (17.1%). Results of the study indicate that individuals above 65 years of age demonstrate increased BC risks as 67(43.2%) men ( $p=0.123$ ) join them with potential disease manifestation (9). Statistical significance regarding this observed pattern was not evident from the analysis results. The prevalence of BC rises in individuals older than 65 because of their weakened immune systems and age-related changes in infection defense functions as risk factors. It was shown that bladder cancer patients exhibited a urine TNF- $\alpha$  level averaging 362.61 $\pm$ 5.76pg /ml whereas controls displayed an average measurement of 352.68  $\pm$  6.75 pg/ml according to [10]. The researchers confirmed a minimum significant level at  $p>0.05$ .

The risk for bladder cancer runs higher among men compared to women because of multiple influencing variables. The

smoking habits of more men combined with their extended exposure to hazardous workplace elements explain the higher incidence of bladder cancer among males [11]. DNA attacks by chemicals lead to genetic material damage in bladder epithelial cells, increasing cancer risk with time [12].

The human biological structure between men and women contributes to these differences. Research indicates that sex hormones, together with their receptors, control bladder cancer development patterns within male patients. The hormone estrogen, which dominates within female bodies, seems to shield bladder tissues from developing cancer through restrictive functions. The male sex hormone known as androgen directly drives bladder cancer development into robust growth patterns. Men receive more bladder cancer diagnoses than women, potentially due to distinct hormonal variables that exist between the sexes [13]. The development of BC depends on proinflammatory cytokines. Inflammatory responses lead to the development of most

malignant tumors while simultaneously influencing tumor initiation progression and metastasis. More than 20% of all cancer cases are associated with ongoing infections and chronic inflammation. Even tumors not originating from chronic inflammation exhibit inflammatory responses, infiltrates, or “tumor-elicited inflammation” in the tumor microenvironment, with increased cytokine expression [14].

TNF plays a significant role in cancer development, which results delete in BC progression. Tumor formation occurs through TNF- $\alpha$ 's role in metalloproteinase release regulation and its ability to stimulate angiogenesis since these features promote cancer cell metastasis. The elevated levels of H<sub>2</sub>O<sub>2</sub> lead to increased TNF- $\alpha$  release as a part of cellular inflammatory processes that contribute to BC transformation [15]. Research showed that tumor tissues expressed elevated TNF- $\alpha$  levels than healthy urothelium. In contrast, tumor growth size, recurrence, and disease progression in stage and grade were associated with elevated TNF- $\alpha$  levels through its angiogenic properties. The TME becomes conducive to bladder cancer cell invasion and migration because TNF- $\alpha$  stimulates MMP-9 secretion [16].

Any pro-inflammatory cytokine status produces a robust response by releasing Interleukin-1 beta (IL-1 $\beta$ ), vital to the body's immune response. Different types of cells, including monocytes, macrophages, and bladder epithelial cells, generate this protein substance in the body. IL-1 $\beta$  remains one of the most potent inflammation-causing agents and shows direct ties to UTI severity [17]. During inflammation, the cell centers its production of IL-1 $\beta$ , yet it maintains strict control systems to minimize tissue destruction. Inflammasome mechanisms activate IL-1 $\beta$  precursor to transform it into its active state. Caspase-1 transforms inactive IL-1 $\beta$  precursor into its mature form, which directs neutrophils and other immune cells toward the site of infection [18].

## **Conclusion**

The research study reveals how pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  establish a link between bladder cancer and UTIs and show their role in urinary bladder cancer development.

## **References**

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