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Original Article

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Association Between Interleukin-6 Levels and Lymph Node Metastasis in Bladder Cancer Patients

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Abstract

Background: Bladder cancer (BC) is one of the urological cancers with high prevalence, mortality, morbidity, and expenditure rates. Urothelial malignancies account for about 90% cases of BC, with squamous and adenocarcinomas making up the remaining 10%. Lymph node metastasis (LNM), the most common type of BC metastasis, is generally found in the pelvic lymph nodes. LNM significantly affects the chances of survival and prognosis for patients with BC. It is feasible to stratify and assess the malignancy of the tumor and its response to therapies using potential tumor markers. Interleukin-6 (IL-6) has been observed to be a predictor of metastasis in lymph nodes in BC. The aim of this study was to evaluate the relationship between IL-6 levels and lymph node metastases in BC patients.

Methods: Thirty-two BC patients between August 2021 and January 2022 were admitted to this study. Data on patient characteristics, clinical data, TNM staging, and IL-6 levels were collected. Univariate analysis was used in the characteristics of the patients.

Results: The total subjects were 32 with 15 results in LNM. The difference in IL-6 levels between the LNM (+) group and the LNM (-) was statistically significant by Fisher's exact test (P = 0.041) and Mann-Whitney U tests (P = 0.003).

Conclusions: The BC patients who had lymph node metastases also had higher serum levels of IL-6.

Keywords: Bladder cancer; Bladder neoplasm; Interleukin-6; Lymph node metastasis

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Introduction

One of the most common urological malignancies is bladder cancer (BC), the 10th most common type of cancer, with expected 213,000 deaths and 573,000 new cases in 2020. Smoking is the main contributor to the increased risk for men and women of developing BC. Urothelial malignancies account for about 90% of BCs, with squamous and adenocarcinomas making up the remainder [1, 2].

Lymph node metastasis (LNM), the most common type of metastatic BC, occurs more frequently in the pelvic lymph nodes. LNM greatly affects the survival and outcome of BC patients. Compared to those without lymph node metastases (T1 90.7%; T2 85%; T3 51%, T4 18%), patients with BC-positive LNM have a substantially worse 5-year cancer-specific survival rate of 27.7%. While computed tomography (CT) or magnetic resonance imaging is commonly used in clinical settings to detect pelvic LNM, it could be difficult to locate metastatic lymph nodes with a diameter less than 6.8 mm [2].

Proinflammatory cytokines are important in the growth of BC [3]. Most malignancies develop as a consequence of inflammatory responses, which also impact tumor initiation, development, advancement, and metastasis. There are 20% or more of all incidences of cancer which are associated with ongoing infections and chronic inflammation; and even tumors that do not occur as a result of chronic inflammation have inflammatory responses infiltrates, or "tumor-elicited inflammation", in the tumor microenvironment with increased cytokine expression [4].

It was initially discovered that the cytokine interleukin-6 (IL-6) can cause B cells to differentiate and mature into cells that produce antibodies. IL-6 can be produced by dendritic cells (DC), B and T lymphocytes, endothelial cells, macrophages, keratinocytes, fibroblasts, and tumor cells. The acute phase reaction of the immune system is highly dependent on IL-6. Acute phase proteins are created in the liver as a result of the initial reaction to local inflammatory stimulation, which is the creation of IL-6. Furthermore, IL-6 is crucial to maintaining the homeostasis of the hematopoietic progenitor cells, hepatocytes, placenta, skeleton, and cardiovascular, endocrine, and neurological systems. IL-6 signaling has been connected to numerous processes. First, the IL-6 receptor (IL-6R) in membrane-bound form is expressed by some subsets of leukocytes, hepatocytes, some epithelial cells. When IL-6 binds, a complex is formed between IL-6R from the plasma membrane and the intracel-

lular signaling component gp130 which then triggers the Janus kinase (JAK) and signal transducer and activator (STAT) signaling through the Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 domain of gp130. STAT3, which is phosphorylated by tyrosine 705, is one of many transcription factors that are phosphorylated when members of the JAK family are activated (pY705). The nucleus receives the phosphorylated form of STAT3, which dimerizes and promotes the production of its target genes. A second pathway is transsignaling, in which IL-6 interacts with cells that express gp130 but not the IL-6R in the extracellular environment to attach to the soluble version of IL-6R. Only DCs that transfer IL-6 to T cells have been reported to engage in a third type of IL-6-induced signaling that is essential for the growth of TH17 cells [5].

In cancer and infections, it is well recognized that IL-6 has pro- and anti-inflammatory effects. When an inflammatory reaction is in its acute phase, tumor necrosis factor (TNF) and IL-1 encourage the expression of IL-6, which causes various components of the acute phase reaction to be overexpressed. Furthermore, IL-6 signaling causes T cells to produce chemokines (including chemokines (CC motif) ligands 4 (CCL4), CCL5, CCL17, and chemokine (CXC motif) ligand 10 (CXCL10)) that support inflammation process. These same mechanisms produce a negative feedback cycle when switching between proinflammatory and anti-inflammatory qualities to reduce inflammation and the immune response. Then, TNFα and IL-1β promote the production of the cytokine signaling inhibitor (SOCS3), which inhibits the expression of target genes for inflammatory IL-6, neutralizing inflammatory IL-6 signaling. Moreover, IL-6 promotes the production of IL-4, differentiating activated T cells into the TH2 phenotype and contributing to the development of an anti-inflammatory environment [5]. The IL-6/STAT3 signaling system, which is active in cancer cells, promotes survival, proliferation of cells, angiogenesis, invasiveness, and metastasis in which carcinogenesis is promoted. IL-6 causes the antiapoptotic gene *Mcl1* and cyclin A to be upregulated in cancer cells, which is one of several signaling pathways that promote tumor survival. Furthermore, IL-6 has been established to promote the production of MMP2 and MMP9, increasing the invasiveness and metastatic potential of cancer cells [5].

IL-6 is upregulated in several malignancies, including melanoma, and higher levels are associated with progression of disease, worse clinical outcomes, and therapeutic resistance [6]. Increase IL-6 was associated with worse prognosis in a number of cancers [7, 8], including pancreatic [9], esophageal [10], melanoma, head and neck squamous cell carcinoma [11], and colon [12, 13]. Furthermore, people with cervical cancer are probably has lower chance of surviving than those whose tumors contain low concentrations of IL-6 [14].

IL-6 showed a positive correlation with the recurrence rate, the clinical staging of BC and is negatively correlated with the survival rate [15]. According to research by Schuettfort et al in 1,036 individuals who underwent radical cystectomy, the presence of higher levels of IL-6 plasma before treatment was associated with a higher probability of lymph node metastases (odds ratio (OR): 1.3, confidence interval (CI): 1.19 - 1.43, P = 0.001) [3].

The objective of this study is to evaluate the correlation between IL-6 levels and lymph node metastases in patients with BC.

Table 1. Patient Characteristics

	N
Gender	
Male	27 (84%)
Female	5 (16%)
Age (mean ± SD)	57.44 ± 13.66
Lymph node metastasis (LNM)	
LNM (+)	15 (47%)
LNM (-)	17 (53%)
IL-6 (mean ± SD)	
LNM (+)	49.76 ± 41.92
LNM (-)	18.53 ± 28.67

SD: standard deviation; IL-6: interleukin-6; LNM: lymph node metastasis.

Materials and Methods

This prospective study was conducted at the Universitas Sumatera Utara Hospital and Haji Adam Malik General Hospital from August 2021 to January 2022. In our study, we used the STROBE cohort reporting guidelines [16]. This study was approved by the Ethics Committee of the Faculty of Medicine of Universitas Sumatera Utara, with the letter number 716/KEP/USU/2021, and was conducted in compliance with the ethical standards of the responsible institution on human subjects as well as with the Helsinki Declaration.

Clinical data and characteristics of the patients were recorded. Adult patients who had been diagnosed with BC were involved in this study. Patients with any other malignancy, other malignancy of upper urinary tract, evidence of acute infection (fever (axillary temperature > 37.2 °C, leukocytosis (≥ 11,000/mm^3)) or missing data were excluded.

After a fast overnight, preoperative serum samples were taken together with plasma samples on the morning of BC surgery day and IL-6 levels were examined. All surgical specimens were analyzed using standard pathological procedures and classified using the TNM staging criteria developed by the American Joint Committee on Cancer in 2017. The incidence of metastasis was determined using a CT scan. Patients were classified as LNM positive (LNM (+)) if node (N) < 1 and LNM (-) if N = 0. IL-6 was then classified as follows: < 7 as low and 7 as high.

Univariate analysis was used for the characteristics of the patient. The Fisher exact test was used in categorical variables and the Mann-Whitney U test in continuous variables. The P value < 0.05 is considered statistically significant.

Results

The Kolmogorov-Smirnov normality test was performed to analyze the distribution of the data with the result that all the data were not normally distributed with a P value < 0.05. (Table 1).

The Fisher exact test showed significant results between IL-6 level and LNM with a P value of 0.041 (Table 2). There

Table 2. IL-6 Level and Lymph Node Metastasis (LNM)

IL-6	LNM		Total	P value[a]
	LNM (-)	LNM (+)		
Low	7	1	8	0.041*
High	10	14	24	
Total	17	15	32	

[a]Fisher exact test results. *Statistically significant. IL-6: interleukin-6.

was a statistically significant distinction between the mean levels of IL-6 of the LNM (+) group compared to the LNM (-) group with Mann-Whitney U test as seen in Table 3.

Discussion

It is widely accepted that inflammation contributes to the development and progression of cancer. Different types of normal cells, immune cells that penetrate tumors, and tumor cells themselves release IL-6. Therefore, it was previously believed that the development of local tumors was responsible for higher levels of systemic IL-6. This study discovered that IL-6 levels were related to lymph node metastases because they were higher in the LNM (+) group compared to the LNM (-) group. This research supports a previous study by Schuettfort et al that identified a significant correlation between increased preoperative plasma levels of IL-6 and a higher incidence of lymph node metastases (OR: 1.3, CI: 1.19 - 1.43, P = 0.001). Furthermore, this study discovered that IL-6 may be useful for identifying individuals who would benefit from more intensive or multimodal treatment and who should be included in prognostic or predictive models. They showed that they could improve the capacity of these models for discrimination and thus guide clinical judgment [3].

Compared to controls, patients with urothelial BC exhibited higher IL-6 levels in serum and urine. BC patients also showed higher amounts of IL-6 in their urine compared to persons with less aggressive nonmuscle-invasive BC. According to this, IL-6 could promote new cancer phenotypes linked to malignancy. A worse prognosis and more metastases were associated with an increase in blood levels of IL-6 in BCs. Protumor effects could be attributed to IL-6, which is the main stimulator of the JAK/STAT3 signaling pathway. STAT3 has an impact on both tumor development and epithelial to mesenchymal transition (EMT), a critical stage in tumor invasion [17]. The EMT is essential for the creation of numerous tissues and organs throughout embryogenesis. EMT is associated with cancer cell survival and resistance to apoptosis, invasion and tumor angiogenesis, advanced tumor metastasis and resistance to treatment, and tumorigenesis, in addition to invasion and tumor angiogenesis [18].

The transcription factor TWIST plays a role in the regulation of EMT. One aspect of smoking's pro-tumorigenic effect on BC is thought to be how it affects TWIST. In addition, IL-6 stimulates angiogenesis and vascular modeling through STAT3n and vascular endothelial growth factor (VEGF), which has an impact on genes that control angiogenesis. BC has a poor prognosis when IL-6 is present. The correlation between IL-6 and the decrease in posttreatment response, decreased survival times, and increased disease failure rates confirm this [17].

Table 3. Association Between IL-6 Level and Lymph Node Metastasis (LNM)

	Mean ± SD		P value[a]
	LNM (-)	LNM (+)	
IL-6	18.53 ± 28.67	49.76 ± 41.92	0.003*

[a]Mann-Whitney U test. *Statistically significant. SD: standard deviation; IL-6: interleukin-6.

Furthermore, some BC patients with muscle invasion also have elevated levels of IL-6. Ho et al discovered in mouse models that STAT3 activation caused carcinoma *in situ* to advance directly to the noninvasive papillary tumor stage, resulting in the formation of invasive malignancy. STAT3 may help tumor cells survive by promoting the production of the antiapoptotic protein Bcl-XL [19]. According to Wu et al, Jak2 and STAT3 were significant contributors to BC cell growth. They found that Jak2 inhibition prevented cells from growing [20].

Proinflammatory cytokines, including IL-6, have been discovered to directly alter DNMT1 expression in several malignancies. Immunohistochemical (IHC) analysis revealed that IL-6 positive samples have a positive connection with nuclear DNMT1 staining. The association between DNMT1 expression and IL-6/STAT3 signaling in BC was investigated to determine if the regulation of IL-6/STAT3 signaling influences DNMT1 expression. Suppression of IL-6 signaling reduced nuclear expression of DNMT1, which was connected to lower levels of p-AKT and p-STAT3, according to the results of the mRNA and protein analyzes. On the other hand, DNMT1 expression was not affected by STAT3 siRNA's direct suppression of STAT3. The mechanism that leads to greater expression of DNMT1 induced by IL-6 has been identified as phosphorylation of Akt kinase. When phosphoinositide 3 kinase/Akt signaling was disrupted with the particular inhibitor LY294002, the reduction in AKT activation was associated with a reduction in DNMT1. Therefore, it was hypothesized that DNMT1 activation in BC was amplified by elevated IL-6 signaling and was mediated by Akt activation [15].

In a study by Chen et al, it was shown that BC tissues produced more IL-6 than nonmalignant cells. Furthermore, muscle-invasive BC was preferentially associated with increased IL-6 staining compared to Ta-T1 illnesses in lower stages. Urine IL-6 levels were significantly higher in individuals with locally progressing bladder transitional cell carcinoma (TCC) compared to those with nonmuscle invasive bladder carcinoma. Therefore, the expression of IL-6 may be connected to a more cancerous phenotype. The clinical fate of BC can be predicted using the cytokine IL-6, which is crucial for the aggressive growth of the disease. The outcome supports the idea that IL-6 is a clinically significant prognostic predictor and may be an effective target for BC therapy [15].

Higher IL-6 levels were also associated with myeloid-derived suppressor cell (MDSC) activity. According to Zheng et al, mice with high amounts of IL-6 exhibited an elevated frequency of MDSCs, which was associated with MDSC prolif-

eration. Those with high MDSC frequencies exhibited a poorer overall survival rate than patients with low MDSC frequencies in myeloid cells. IL-6 increased MDSC proliferation and improved its ability to suppress the immune system by triggering the MAPK signaling system, both of which helped MIBC advance. Furthermore, those with high amounts of IL-6 were at increased risk of lymphatic and distant metastases [21].

Our study limitation was that the sample size appeared to be small, but this was consistent with the incidence of BC in our region. Although the size of our patient sample was limited, these findings were considered important due to the prospective study design. In addition, the metastasis was determined with CT scan, leading to difficulties in locating metastatic lymph nodes with diameters less than 6.8 mm. More studies are needed with a larger sample size.

Conclusions

The BC patients who have lymph node metastases tend to have higher blood levels of IL-6. The evidence for IL-6 participation in BC development has been reinforced by several ways of raising IL-6 that have been postulated. IL-6 is a clinically significant prognostic predictor and may be an effective target for BC therapy. More detailed studies of benefits in real world settings are still needed, as is a cost-effectiveness study, before IL-6 may be used in clinical practice.

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Conflict of Interest

All authors of this study have nothing to disclose.

Informed Consent

Written informed consent was obtained from the patients for publication of this work.

Author Contributions

SMW, FFP, DISS, WSW contributed equally to the study conception, design, material preparation, data collection, data analysis, manuscript drafting, and manuscript writing. SMW is the guarantor of this work. All authors have read and approved the final manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations

BC: Bladder cancer; DC: dendritic cells; EMT: epithelial to mesenchymal transition; JAK: Janus kinase; IL-6: interleukin-6; IL-6R: IL-6 receptor; LNM: lymph node metastasis; MDSC: myeloid-derived suppressor cell; MIBC: muscle invasive bladder cancer; NIMBC: nonmuscle invasive bladder cancer; SH2: Src homology region 2; STAT: signal transducer and activator

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