EVALUATION OF INTERLEUKIN-6 (IL-6) AS A TUMOR MARKER FOR URINARY BLADDER CANCER

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ABSTRACT

Interleukin-6 is a multifunctional cytokine that involves in the activation of different intra-cellular signalling pathways resulting in the regulation of inflammation, immune response, proliferation and differentiation of tissue etc. IL-6 acts by means of two type of pathways namely classical signalling and trans-signalling according to the availability of it receptors, membrane bound IL-6R or soluble IL-6R respectively. Increased circulation levels of IL-6 have been associated with several patho-physiological conditions including cancers. In this review article, we have compiled available studies regarding IL-6 and its role in urinary bladder cancer. Most of the studies found an association between increased levels of IL-6 and its positive staining in advance stages of urinary bladder cancer furthermore cell-line, animal model and genetic based studies confirms its role in urinary bladder cancer. Hence, after understanding its definite role in urinary bladder cancer, IL-6 can be used as a prognostic marker. Urinary bladder cancer growth may be controlled by using anti-IL-6 monoclonal antibody against IL-6 as a therapeutic mean or by using soluble gp130 receptor which inhibits its trans-signalling pathway. The area of interest is to find out its applicability in nature of disease, its progression and to design newer drugs which are curative as well as disease preventive. IL-6 has dual role as pro-inflammatory and anti-inflammatory agent, it is expected that it can promote cancer and can also control it, therefore there is need to discover the specific point where it has a noble activity.

KEYWORDS: Urinary bladder cancer, cytokines, interleukin-6.
INTRODUCTION
Cytokines regulate many functions of cells via activating or deactivating different pathways. Their concentration differs by a wide range in normal and pathological conditions. Interleukin-6 (IL-6) is one of the cytokines that plays an important role in the cell physiology.\(^1\) IL-6 is a multifunctional cytokine that influences various cellular processes including immune response, regulation of proliferation, differentiation and inflammation within local region or elsewhere in the body. It is encoded by IL-6 gene present on 7p15-p21 chromosome, the transcript is 26kDa protein composed of 184 amino acids.\(^2\) Secretion of IL-6 is stimulated by IL-1, INF, TNF, lypo-polysaccharide, DNA viruses and RNA viruses. IL-6 is majorly produced by macrophages and monocytes whereas a minor amount is also formed by fibroblast, endothelial cells, amnion cells, T and B lymphocytes and chondrocytes.\(^3\) IL-6 is normally present in picomolar (10\(^{-12}\) M) concentration, however its level increases up to 100-1000 fold in pathological conditions such as infection, trauma or inflammation by infection, tumorigenesis or by other diseases. So researchers are interested to know about it specific role in tumorigenesis in urinary bladder cancer and looking for specific urinary bladder cancer marker but till date no specific marker have been discovered.

Several experimental and clinical studies have linked the cytokines and their role in different cancers. Various pro-inflammatory cytokines are released by immune cells and regulate cancer cell growth therefore contribute to tumor invasion and metastasis. Among these, IL-6 seems to play a central role and involves in tumor growth, cancer cell differentiation and change in microenvironment which further results in neo-angiogenesis, inhibited apoptosis and acquired cell defence.\(^4\) An increased level of IL-6 has been measured in different cancers including bladder prostate, colon and breast cancer.\(^5\text{-}^7\) IL-6 along with other cytokines either in autocrine or paracrine manner involves in invasion and metastasis of cancer. IL-6 is a major activator of STAT-3 signalling pathway which further maintains constitutive NF-κB. NF-κB involves in gene regulation responsible for cell proliferation and cell survival. Therefore defects in IL-6 may result in altered functions of NF-κB and ultimately be responsible for cancer development. Epithelial cells in carcinogenic area showed lack of mbIL-6R and an increased amount of ADAM17 (a disintegrin and metalloproteinase 17) responsible for the cleavage of mbIL-6R.\(^8\) Cleavage of mbIL-6R accompanied by formation of IL-6/sIL-6R complex hinders classical signalling of IL-6 on epithelial cell. Upon the stimulation of IL-6/sIL-6R complex T-cells become resistant to apoptosis and corroborate growth of tumor.\(^9\) Therefore, considering the point that IL-6 trans-
signalling pathway promotes tumor growth that may be inhibited by stimulating agent that hinders or slows down the pathway of trans-signalling activity of IL-6.\textsuperscript{[10]}

**Mode of action:** On targeted cell, IL-6 firstly binds with IL-6 receptor (IL-6R) forming an IL-6/IL-6R complex. This complex further binds with two molecules of signal transducing protein glycol protein (gp130) leading to the activation of different intracellular signalling pathways.\textsuperscript{[11]} The gp130 protein is present as preformed inactive dimers and is expressed by most of the cells in body.\textsuperscript{[12,13]} IL-6R is also preformed dimer but depending on its availability IL-6 protein acts via two type of signalling pathways i.e. classical-signalling and trans-signalling. **Classical signalling** involves activation of cell by binding of membrane bound trans-signal protein receptor (mbIL-6R) and IL-6, forming IL-6/mbIL-6R complex which further initiates a physiological response when combined with cell that expresses gp130 receptor. The mbIL-6R is expressed by only a few types of cells such as macrophages, hepatocytes, neutrophils and some type of T-cells.\textsuperscript{[14,15]} The process of activation of cell by binding of IL-6 with a soluble IL-6 receptor (sIL-6R), found in various body fluids, and forming IL-6/sIL-6R complex which stimulates cells representing gp130 receptor, is known as **trans-signalling.** Formation of sIL-6R occurs by proteolysis of mbIL-6R or transcription of alternative spliced mRNA lacking transmembrane and cytosolic domain.\textsuperscript{[16,17]} The sIL-6R can stimulate cells representing both IL-6 and gp130 on the same cell or cells representing IL-6 and gp130 on different cells thus it is responsible for autocrine as well as paracrine activity. A soluble gp130 (sgp130) has also been detected in plasma which binds to IL-6/sIL-6R complex and affects trans-signalling activity of IL-6\textsuperscript{[18,19]} leading to pro-inflammatory response in tissue. Activity of IL-6 is presented in flow chart **Figure 1.**

**Search methods:** We performed an article search through Pub Med, Google Scholar, and Medscape using key words interleukine-6, urinary bladder cancer, cancer angiogenesis, and cytokines. All types of IL-6 related ones articles were included and studied and relevant were compiled.

**Role of IL-6 in urinary bladder cancer:** In urinary bladder cancer, it seems that IL-6 contributes to tumor proliferation and differentiation, and that is why the study of the mechanism by which it affects cancer biology is presently a hot topic of research. The correlation between polymorphism in IL-6 gene and risk of urinary bladder cancer has not been extensively studied, however, some of such studies are summarised in **Table 1.** Various studies done in urinary bladder cancer to evaluate the role of IL-6 can be classified as below.
In-vitro or in-vivo studies: In their study to understand the role of IL-6 in urinary bladder cancer, K. Esuvaranathan et al 1995 found that during the BCG treatment both the epithelial cell of urinary bladder and the adjacent leukocytes stained for IL-6 which established its involvement with urinary bladder cancer.

Later to understand the association of IL-6 with urinary bladder cancer, an in-vitro study by Ke-Hung Tsui, 2013, involving T-24 cell line (IL-6 knockdown experiment) and HT1376 cell line (IL-6 overexpression experiment), showed that IL-6 reduces cell proliferation, differentiation and migration in IL-6 over-expressed cell line. In in-vivo study, xenograft animal showed that over-expression of IL-6 reduces tumorigenicity and in IL-6 knockdown animal an accelerated tumorigenesis was found. While in the same year 2013, Chen M-F et al. observed, in human bladder cancer cell line, an overexpression of IL-6 in urinary bladder cancer specimens as compared to non-malignant specimens both in mRNA and in protein levels. Thus IL-6 knockdown results in decreased cell proliferation, less epithelial-mesenchymal transition (EMT), decreased DNA methyltransferase-1 expression and attenuated angiogenesis and hence is responsible for attenuated tumor growth and invasive capability of cancer.

Biochemical studies: In an earlier study, K. Esuvaranathan et al 1995 measured IL-6 level in the urine samples of the urinary bladder cancer patients and found an increased level in CIS bladder cancer patients which further increased when treated with BCG treatment. Furthermore, in a prospective study, Tommaso Cai et al. 2007 observed ratio of IL-6 and 10 in urine samples at different intervals before and after BCG treatment and found a significant difference with 0.83% sensitivity and 0.76% specificity of the ELISA test. However, the number of patients included in the study was very low. Recently Chen MF 2013 studied that IL-6 level in urine sample of urinary bladder cancer patients was significantly higher in stage T3-T4 as compared to ≤T2 stages. In a study by Ben Andrews et al, 2002 in urinary bladder cancer patients higher levels of IL-6 were observed as compared to healthy controls and these levels were found to be associated with cancer stage and progression. Contradictorily, a study by Nidhal Abdulmohymen, et al, 2010 observed that serum IL-6 level was statistically insignificantly higher in cancerous patients as compared to non-cancerous patients.

Histopathological studies: Naik DSL et al, 2011 demonstrated an Immunohistochemical (IHC) study of bladder cancer tissue with IL-6 staining and found 80% cases positive with cytoplasmic staining. They found no correlation with the expression of epithelial growth
factor receptor (EGFR) and IL-6 staining. Chen MF, 2013 found that IHC for transitional cell carcinoma (TCC), indicate 51% positive staining for stages T2-T4 and 65% staining for more advanced cancer but the cancer in T1 or CIS expressed only 17.5% staining.[22]

**Genetic studies:** The first genetic study of IL-6 was done by Dan Leibovici et al. 2005, who found that the C/C genotype of IL-6-174G>C was associated with an increased risk of urinary bladder cancer.[27] With the same findings Dinesh Ahirwar, 2008 concluded that low producing C/C variant of IL-6-174G>C favours Th1 response and may be a good prognostic indicator for the treatment and survival of BCG treated urinary bladder cancer patients.[28] In a study, Chia-Chang Wu et al. 2013 investigated the joint effect of polymorphism in inflammation genes including IL-6, interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-alpha) and Arsenic profile with urinary bladder cancer risk and observed a correlation of TNF-alpha and IL-8 polymorphism with urinary bladder cancer risk, but were not able to explain the relationship with IL-6 polymorphism because IL-6 polymorphism was not fitted in the Hardy–Weinberg equilibrium (HWE).[29]

Sparse literatures are available to understand the association of IL-6 with urinary bladder cancer, therefore its function in urinary bladder cancer is still unclear. As IL-6 is actively involved in the pathogenesis and progression of different malignancies since it plays a central role in immune response against tissue injury, an increased level of IL-6 had been observed in cancer patients[9,10] including urinary bladder cancer patients.[30] IL-6 promotes tumor growth via inhibiting apoptosis and promoting angiogenesis[6], IL-6 promotes proliferation of cancer especially in advance stage. Therefore current researches are focused to explore the in-depth knowledge of IL-6 and its use as a prognostic marker in urinary bladder cancer.[31] Different studies have associated inflammation with the development of cancers.[32-34] Inflammation involves as a major agent in the etiology of different cancers and IL-6 plays a central role in the inflammation therefore regular use of anti-inflammatory agents like aspirin reduces the risk of cancer.[33] It is supposed that anti-inflammatory responses of IL-6 are mediated by classical signalling while pro-inflammatory activities of IL-6 are mediated by trans-signalling.[33] Although in present time IL-6 blocking agents block both the signalling pathways and therefore there is a need of specific trans-signalling inhibitor for the betterment of public health.[31] Trans-signalling pathway of IL-6 is accomplished by binding of IL-6/sIL-6R complex with membrane bound gp130 protein receptor, however in the presence of soluble gp130 protein receptor in plasma IL-6/sIL-6R complex bind with sgp130 in
circulation itself and does not let the complex to reach cell membrane and thus inhibit trans-signalling pathway. Because of its selective antagonistic activity for trans-signalling pathway of IL-6, sgp130 may be used as a therapeutic agent for chronic inflammatory disease including cancer.\textsuperscript{[35]} Thus opening a window in drug designing to treat these conditions, Tocilizumab, an anti-IL-6R monoclonal antibody (mAb) has been approved for the treatment of inflammatory diseases. The use of mAb against IL-6 reduces levels of CRP remarkably; hence mAb may be useful in the treatment of cancer patients at the early stages and can correlate with survival.\textsuperscript{[36]} Zhang G, 1999 observed that concentration of IL-6 in plasma was higher in less survived breast cancer patients in comparison to the more survived breast cancer patients. As plasma level is higher in advance stage cancers it promotes its use as prognostic marker. Further, it supports the hypothesis of developing anti-IL-6 drugs to decelerate the disease prognosis.\textsuperscript{[37]}

Further research is needed to figure out genetic and epigenetic role of IL-6 in urinary bladder cancer and evaluate its role as a prognostic marker in urinary bladder cancer patients. For a better understanding there is a need of prospective studies, multi-centric studies with large sample sizes and follow-up including all the risk factors, histological grading and staging of tumor in relation to IL-6 and cancer development. The area of interest is to know its applicability in nature of disease, its progression and to design newer drug which are curative as well as disease preventive. Finally, as IL-6 has dual role as pro-inflammatory and anti-inflammatory agent, it is expected that it can promote cancer and can also control it, therefore there is need to discover the specific point where it has a noble activity. As on date, most of the studies that have been done are limited to understanding the relationship between IL-6 gene and urinary bladder cancer, but in order to learn more about- the relationship, there is a requirement of exploring the association of all the major genes of inflammatory pathway with development and proliferation of cancer and constructing a haplotype analysis to confirm their affective role and extend the findings. Functional analysis shall be done to measure phenotype expression and evaluate genotype and phenotype correlation in the context of urinary bladder cancer development. Further studies are needed to understand risk association and their correlation with urinary bladder cancer.
Table 1: Interleukine-6 associated urinary bladder cancer studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Subject</th>
<th>Study type</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milosevic Radovan, 2011</td>
<td>Urine</td>
<td>ELISA</td>
<td>IL-6 level was higher in advance bladder cancer than superficial.</td>
<td>[38]</td>
</tr>
<tr>
<td>Miao-Fen Chen, 2013</td>
<td>Tissue</td>
<td>In-vivo, in-vitro, IHC</td>
<td>Knockdown IL-6 responsible for attenuated tumor growth and invasive capability.</td>
<td>[22]</td>
</tr>
<tr>
<td>Dan Leibovici, 2005</td>
<td>Blood</td>
<td>Genetic</td>
<td>A significant relationship between polymorphism and risk of urinary bladder cancer</td>
<td>[27]</td>
</tr>
<tr>
<td>Ke-Hung Tsui, 2013</td>
<td>UBC</td>
<td>In-vivo and in-vitro</td>
<td>Overexpression of IL-6 reduces proliferation, differentiation and tumorigenesis.</td>
<td>[21]</td>
</tr>
<tr>
<td>Naik DSL, 2011</td>
<td>Tissue</td>
<td>IHC</td>
<td>IL-6 responsible for Cytoplasmic staining with 80% positivity.</td>
<td>[26]</td>
</tr>
<tr>
<td>Dinesh Ahirwar, 2008</td>
<td>Blood</td>
<td>Genetic</td>
<td>A good prognostic indicator for the treatment of BCG treated bladder cancer patients.</td>
<td>[28]</td>
</tr>
<tr>
<td>Nidhal Abdulmohyem, 2010</td>
<td>Serum</td>
<td>Genetic</td>
<td>An increase in IL-6 level due to bacterial infection although patients are with or without urinary bladder cancer.</td>
<td>[25]</td>
</tr>
<tr>
<td>K Esuvaranathan, 1995</td>
<td>Urine, tissue</td>
<td>IHC, ELISA</td>
<td>Elevated level of IL-6 during BCG treatment may caused by urothelial cell as well as by leukocytes</td>
<td>[20]</td>
</tr>
<tr>
<td>Tommaso Cai, 2007</td>
<td>Urine</td>
<td>ELISA</td>
<td>IL-6/10 ratio may use for predicting intermediate risk superficial bladder carcinoma recurrence.</td>
<td>[23]</td>
</tr>
</tbody>
</table>

**Figure 1.** Flow chart of IL-6 showing its activity and cellular effects.

IL-6 Simulated by
- IL-1, INF, TNF, viruses, lypo polysaccharide

IL-6 Produced by
- Majority-macrophages, monocytes
- Minorly- endothelial cell, B & T lymphocytes, fibroblast

Bind with
- Classical-signalling
  - By mIL-6R

Bind with
- Trans-signalling
  - by eIL-6R

Activation of gp130
- Complex formation activates cellular signalling pathways
  - Results in immune response, inflammatory responses, proliferation, malignant differentiation, regulation of tumor, neo-angiogenesis.
REFERENCES


