
Serum interleukin-6 level using ELISA in patients with bladder cancer and having urinary tract infection

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Abstract:

Background: IL-6 blood levels are elevated in numerous infectious, inflammatory, and autoimmune diseases and in cancer in association with increased synthesis of other cytokines stimulated by infection

Aim: This study carried out to shed light on possible role of IL-6 in bladder cancer and its correlation with bacterial infection.

Materials & Methods: Serum IL-6 levels were measured in 132 patients and 25 healthy subjects by using ELISA technique. The patients were divided into 3 groups :Group-1: Newly diagnosed bladder cancer patients, Group-2: Post-chemotherapy patients, and Group-3: Other bladder disorders rather than bladder cancer. Urinary tract infections were studied for all groups by culturing urine samples using selective culture media.

Results: There was no significant elevation of mean serum IL-6 levels between different groups ,but there were higher levels in cancerous patients ,when compared with non-cancerous patients, and the mean serum IL-6 levels in bladder cancer patients in correlation to the tumor grade ,showed no significant difference, but there was significant difference of serum IL-6 levels with mean value of (5903.8765 pg/ml) in 34 Gram-negative bacterial infected patients with and without bladder cancer, and (8466.6667 pg/ml) in 3 Gram-positive bacterial infected patients with and without bladder cancer than those who were free of infection with P value< 0.001 .

Conclusion: Bacterial infected patients with and without bladder cancer revealed a significant increase in the mean serum IL-6 levels by ELISA.

Key words: IL-6 .serum IL-6.bladder cancer.UTI .ELISA.

Introduction

Epithelial cells lining the urinary bladder mucosa are engaged in numerous functions that act in concert to prevent exposure of the sensitive upper urinary tract to bacteria.

The epithelial surfaces that line mucosal compartments traditionally have been considered barriers to pathogen entry, and several studies, primarily *in vitro*, have demonstrated that epithelial cells can produce cytokines, chemokines, and antimicrobial peptides after bacterial stimulation^[1]. Interleukine-6 (IL-6) is a pleiotropic cytokine with varied systemic functions^[2]. IL-6 is secreted by a number of different cell types, and IL-6 blood levels are elevated in numerous infectious, inflammatory, and autoimmune diseases and in cancer in association with increased synthesis of other cytokines stimulated by infection, trauma, and immunological challenge^[3-5].

The physiological activity of IL-6 is complex, producing both pro-inflammatory and anti-inflammatory effects in the immune system. IL-6 modulates the transcription of several liver-specific genes during acute inflammatory states, particularly C-Reactive protein (CRP), and controls the survival of normal plasmablastic cells, as demonstrated in reactive plasmacytosis using monoclonal antibodies (mAbs) directed against IL-6^[6,7]. In addition, IL-6 is an activator or an inhibitor of T-cell responses depending on the target and the system used *in vitro*. This interaction of pro-inflammatory and

anti-inflammatory activities suggests that IL-6 may play a role in regulating the physiological response to disease^[8]. Increased production of IL-6 has been implicated in various disease processes, including bladder cancer^[9].

In some instances, IL-6 is implicated in proliferation pathways, because it acts with other factors, such as, heparin-binding epithelial growth factor and hepatocyte growth factor^[9,10].

During the late stage of tumor growth, tumor expression is associated with an increase in the levels of IL-1, IL-6, and acute-phase proteins^[11,12,13].

It has been previously suggested that interleukin-6 plays a significant role in bladder carcinoma. To improve this, Okamoto and Oyasu,(1996)^[14], conducted an experiment to determine whether over expressed IL-6 enhanced the tumorigenicity of a weakly tumorigenic rat bladder carcinoma, and whether it was sufficient to induce a tumorigenic phenotype in a non-tumorigenic, anchorage-dependent rat urothelial cell line.

They concluded that acquisition of the ability to synthesize endogenous IL-6 markedly accelerates the growth rate of weakly tumorigenic rat urothelial cells.

This study was carried out to estimate serum IL-6 level in patients with and without bladder cancer, and estimate its frequency in bacterial infected and non-infected patients, to correlate between bladder cancer and bladder bacterial infection in this regard

Materials & Methods

This study included 132 Iraqi patients with bladder cancer and other urological disorders. They were divided into three groups, in which 69 (43.9%) patients were newly diagnosed for bladder cancer (51 (73.9%) male, and 18 (26.1%) female)(group1), 27 (17.2%) patients with bladder cancer and had been received chemotherapy treatment(20 (74.1%) male, and 7 (25.9%) female)(group2), 36 (22.9%)

patients with different bladder disorders other than bladder cancer: Chronic bilharzias cystitis, Chronic non-specific cystitis, Dysplasia, and Mild non-specific cystitis (24 (66.7%) male, and 12 (33.3%) female)(group3) as patients control, and there were 25 (15.9%) apparently healthy individuals(17 (68.0%) male, and 8 (32.0%) female)as healthy control,(**Table -1**).

Table 1: Subjects included in the study.

Gender	Group				Total
	Group-1 Newly diagnosed	Group-2 Post- chemotherapy	Group-3 Patients control	Group-4 Healthy subjects	
Male No.	51	20	24	17	112
% of Total	(73.9)	(74.1)	(66.7)	(68.0)	(71.3)
Female No.	18	7	12	8	45
% of Total	(26.1)	(25.9)	(33.3)	(32.0)	(28.7)
Total No.	69	27	36	25	157
% of total	(43.9)	(17.2)	(22.9)	(15.9)	(100.0)

Blood samples: Three ml of blood were collected by venipuncture, using plastic disposable 5ml syringes, from all patients and control groups. Blood samples were allowed to clot at room temperature, then centrifuged for 15 minutes at approximately 500 rpm to obtain serum. Serum samples were stored aliquots at -20 °C until used for the measurement of interleukine-6 (IL-6) levels.

Urine samples were collected before cystoscopy or surgery for patients of groups 1, 2, 3 and for healthy subjects, as aseptically as possible, in sterile containers. The collected mid-stream specimens were transported to the laboratory within 30 minutes of the collection and cultured on a specific media for bacterial isolation.

Determination of Serum Interleukin-6(IL-6):

Serum level of IL-6 were measured by mean of enzyme immunoassay using ELISA kit (Mabtech AB, Sweden), based upon coating wells of a high protein binding ELISA plate with monoclonal antibody specific for human IL-6 13A5 .Samples or

standards were pipette into these wells, followed by the addition of a biotinylated second antibody . During the first incubation, the IL-6 antigen binds simultaneously to the capture antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, streptavidin-peroxidase is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove the entire unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of human IL-6 present in the original specimen (Goldsby *et al.*, 2000)^[15].

Statistical analysis:

ANOVA test, Student's *t* test, chi-square (χ^2) test of significance was adopted for the comparison and calculation of association in qualitative data. Correlation coefficient was used as a qualitative indicator to express the relative relation between

variables (Markers) and to measure the dependence of one variable on the other.

Results:

One hundred and twenty three sera samples of different study groups were analyzed by indirect ELISA method for assessment of IL-6 levels.

The descriptive statistic of the sera levels of IL-6 in the four study groups were shown in **Table 2** and **figure 1**.

ANOVA test was used to detect any significant correlation in serum IL-6 levels between these four groups, and the results showed that there was no significant difference neither between groups nor within groups (F test = 1.876, $P > 0.05$).

Table 2: Descriptive differential analysis and differences in median levels of serum IL-6 among groups of patients with and without bladder cancer included in the study.

Variable	Group	No.	Mean	Median	Range	Min.	Max	F test P value
Serum IL-6 (pg/ml)	Group-1	51	3693.6049	3200.0000	9999.92	0.08	10000.00	F test
	Group-2	17	4972.7424	6400.0000	9999.20	10000.00	1.876	8200.00
	Group-3	19	3002.4032	830.0000	0.80	8199.20	8199.90	
	Group-4	16	2138.2775	5.0000	0.80	8200.00	0.10	

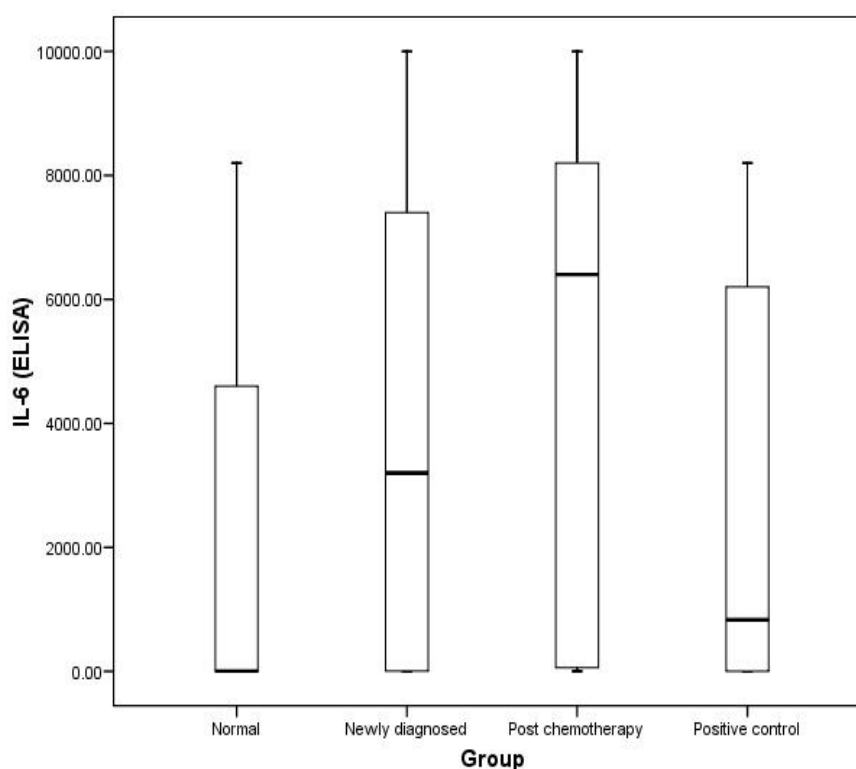


Figure 1: Median levels of serum IL-6 among groups of patients with & without bladder cancer and the other subjects included in the study.

Correlation of serum Interleukin-6 to Bladder Cancer:

There was an elevated mean serum level of IL-6 in sera of bladder cancer patients (4013.3893 pg/ml), when compared to serum IL-6 levels of non-cancerous patients (2607.3743 pg/ml), but without statistical significance ($P = 0.052$), **Table 3**.

Descriptive statistical analysis of serum IL-6 in bladder cancer patients in correlation to the tumor grade:

Figure 2 shows the relationship of serum IL-6 median level to tumor grade of bladder cancer patients, and differences in median among them.

The highest IL-6 levels were observed in sera of bladder cancer patients with grade-3(4982.2727 pg/ml), then in sera of bladder cancer patients with grade-1(4032.5816 pg/ml), and then grade-2(

3730.7876 pg/ml). The results showed no significant difference in sera IL-6 levels between groups, or within groups

Table 3: Mean serum Interleukin-6 levels in patients with and without bladder cancer.

Variable	Type-CA	No.	Mean	Median	Range	t test, P value
Serum IL_6 Level	Cancer	68	4013.3893	3800.0000	9999.92	t- test, P-value 1.976 0.052
	Non-Cancer	35	2607.3743	830.0000	8199.20	

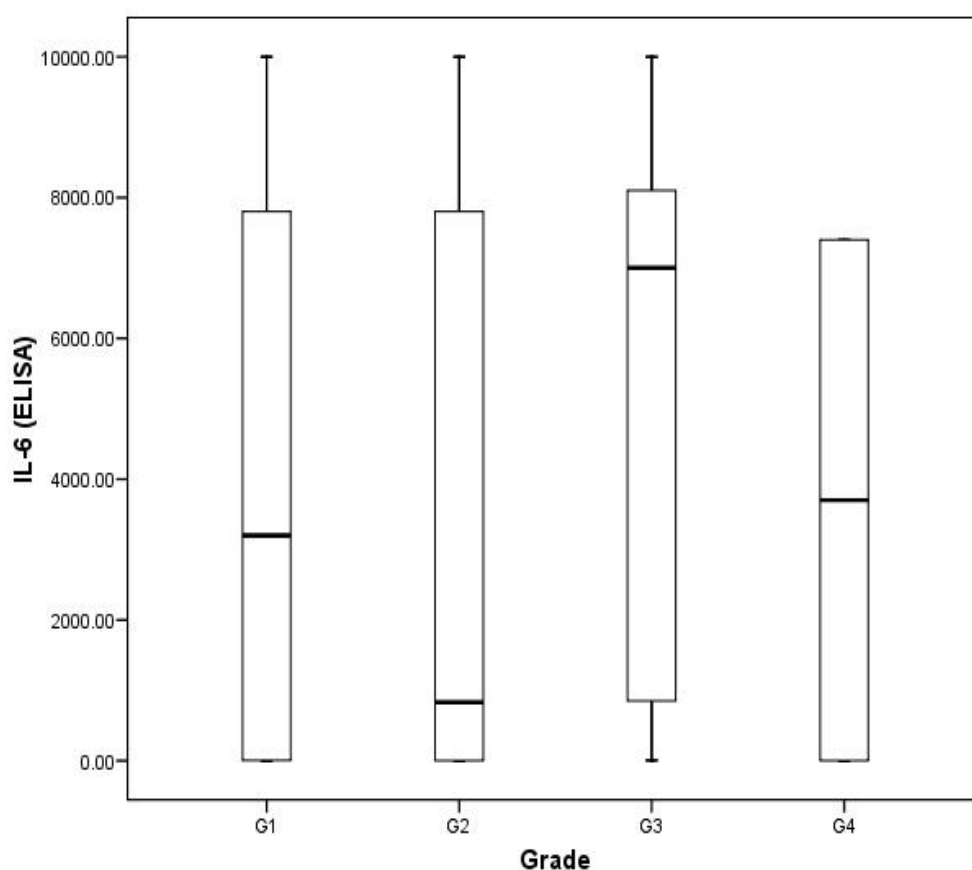


Figure 2: Median of Serum IL-6 Levels in Bladder Cancer Patients in Correlation to the Tumor Grade

Serum IL-6 in Patients With & Without Bladder Cancer in Correlation to Bacterial Infection:

Thirty four out of 103 patients with bladder cancer and other urological diseases ,showed positive urine culture for Gram-negative bacteria with mean serum IL-6 level(5903.8765 pg/ml), while only 3 out of 103 patients gave positive urine

culture of Gram-positive bacteria with mean serum IL-6 level (8466.6667 pg/ml).

It was found that serum IL-6 level by ELISA significantly associated with both type of bacterial infection (Gram-Positive & Gram-Negative bacteria) ($P < 0.01$), figure 3.

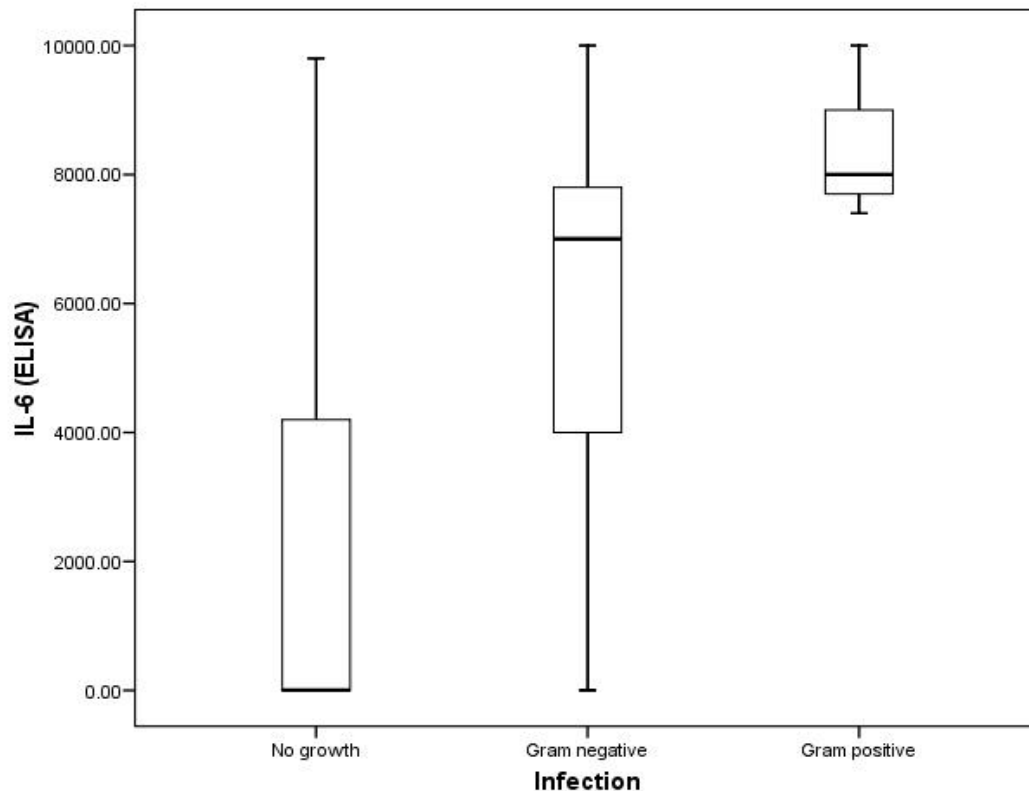


Figure 3 Differences in median of serum IL-6 Levels in patients with and without bladder cancer in correlation to bacterial infection

Discussion

Highest serum IL-6 mean levels were observed in post-chemotherapy bladder cancer patients without any significant difference, neither between groups, nor within groups, and there was an elevated mean serum level of IL-6 in sera of bladder cancer patients, when compared with non-cancerous patients. It has been previously suggested that IL-6 plays a significant role in bladder carcinoma by the ability to synthesize endogenous IL-6 that can markedly accelerates the growth rate of weakly tumorigenic urothelial cells, but is not sufficient to induce a tumorigenic phenotypes in non-tumorigenic cells^[14].

In the majority of studies, active disease is associated with elevated serum levels of IL-6, which are related to disease severity and outcome^[5]. Also, Okamoto *et al.*, (1997)^[16], demonstrated that IL-6 functions as an autocrine growth factor for bladder carcinoma cells, but not for normal urothelial cells and that it may be a factor accounting for the marked enhancement of inflammation-associated bladder carcinogenesis and tumor growth. At the time that tumor expression is associated with an increase in the levels of IL-6 during the late stage of tumor growth^[12,17], the results of serum IL-6 levels in this study, showed no differences between different tumor grades, which might be associated with the

presence or absence of IL-6R on the tumor cells in different tumor grades.^[13]

Regarding bacterial infections in patients with and without bladder cancer, there was a significant association between serum IL-6 level detected by ELISA and each type of bacterial infection (Gram-positive and Gram-negative bacteria), and this agreed with^[4,5], whom indicated that IL-6 blood levels are elevated in numerous infectious, inflammatory, and autoimmune diseases and in cancer in association with increased synthesis of other cytokines stimulated by infection, trauma, and immunological challenge.

Conclusion:

There was an elevated mean serum level of IL-6 in sera of bladder cancer patients, when compared with non-cancerous patients, but there was no significant difference in its level in correlation with the tumor grade, and bacterial infected patients with and without bladder cancer revealed a significant increase in the mean serum IL-6 levels by ELISA. So, IL-6 is not specific for bladder cancer.

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