

## Research Article

# Immunological Estimation of Inflammatory Interleukins (IL-4, IL-6 & IL-10) Among Patients with Bladder Cancer

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### Abstract:

The aim of this study was to determine Immunomolecular expression of inflammatory interleukins (IL-4, IL-6 & IL-10) by using ELISA, conventional PCR and Sequencing technologies, to give acknowledge about the roles of these interleukins in patients with bladder cancer in Basrah province. A case-control study included 85 confirmed bladder cancer patients and 80 individual as a control group. Data about age, gender, smoking, alcohol drinking, family history, occupation, residency and clinical findings for all patients with urothelial carcinoma were collected. This study show the effect of demographical factors ( age, gender smoking, family history, alcohol drinking, occupation and residency) in patients with bladder cancer. The current study measure interleukins (IL-4, IL-6 & IL-10) concentration (pg/ml) by using ELISA technology among various age groups, gender, tumor sages (T1-T4) and case status (newly diagnosed, advance & recurrence). The results show significant increase of interleukins 4, 6 and 10 in comparison with control group (P-value= 0.008, 0.009, 0.001) respectively.

### Introduction

Urothelial carcinoma is a disease in which the lining of the bladder lose their ability to regulate their growth and begin to divide uncontrollably, this abnormal growth can form a tumor, This abnormality may be caused by secondary chronic inflammation of lower urinary tract, stones, smoking and exposure to various chemicals, exposure to carcinogens products and compounds, and secondary schistosomiasis (Cohen *et al.*, 2000). Bladder cancer is a heterogeneous either, low-grade, superficial papillary lesion or high-grade, invasive tumor that usually has metastasized at the time of presentation (Ferri, 2003).

Transitional cell carcinoma accounts for almost 5% of all human cancers and represents 95% of all urothelial tumors (Ashoor, 2007). It is the second most common tumor of the genitourinary tract, it's also the second most common cause of death from these cancers (Williams *et al.*, 2001). It is the most common malignant tumor in the Western countries and the fifth most common cancer among males with an incidence of 29.8 per 100.000 males per year (Ashoor, 2007).in addition, it is the most common malignant tumor in the Middle East and Africa where Schistosomiasis is a prevelant problem (Kadhim, 2009). In Iraq it's the third most common malignant tumor with incidence 6.6% in both males and females reported by Iraqi cancer registry (ICR), It's the second most common tumor in males (10.3%) and the eighth in females (3%)(ICR, 2000) .

The pathogenesis and transition of normal urothelium to bladder carcinoma are multifactorial processes, Chronic inflammation causes initiation and progression of the underlying pathophysiology of aggressive invasive and

metastatic tumor. A dichotomy is observed in the role of immune cells in bladder carcinoma. although the immune response protect the host by inhibiting neoplastic growth, several immune cells, including neutrophils, macrophages and T-lymphocytes, promote the development and progression of tumors.

The development of an effective immune response involves lymphoid cells, inflammatory cells, and hematopoietic cells, The complex interactions among these cells are mediated by a group of protein designated (Cytokines) to denote their role in cell- to cell communication, they are secreted in extremely low concentrations, and most of them manifest their biological effect through specific receptors, with high binding affinities, expressed at the surface of their target cells. (AL-khatib, 2007).

Interleukin-4 (IL-4), a member of the  $\alpha$ -helical cytokine family, is produced by activated CD4+ T cells, basophils, and mast cells. IL-4 is the central differentiation factor driving Th2 development, eliminating extracellular pathogens, and inhibiting Th1 differentiation. Therefore, IL-4 plays an important role in surveillance and elimination of transformed cells . Numerous epidemiologic studies have examined the association of IL-4 gene polymorphisms with cancer risk ( Muller-Hermelink, *et al.*, 2008). Moreover IL-4 receptor (IL-4R) as a heterodimeric complex can bind to the Th2 cytokines IL-4 and IL-13, High level expression of IL-4R has been observed in colorectal carcinoma, In addition, polymorphisms of IL-4R were involved in the etiology of various cancers, including pancreatic cancer, bladder cancer and cervical cancer (Luo, *et al.*, 2015)

Interlukin-6 is a multifunctional cytokine that involves in the activation of different intra-cellular signaling 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 signaling and trans-signaling 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, Most of the studies found an association between increased levels of IL-6 and its positive staining in advance stages of urinary bladder cancer(Gautam, *et al.*, 2015).

IL-10 functions as a Th1 inhibitory cytokine in bladder cancer, Studies have revealed that patients with bladder cancer develop a Th2 dominant status with a deficient Th1 immune response, Increased levels of IL-10 and other Th2 cytokines (e.g., IL-4, IL-5 and IL-6), along with decreased levels of Th1 cytokines (e.g., IFN- $\gamma$  and IL2), have been observed in the serum of bladder cancer patients (Satyam, *et al.*, 2011). This circulating cytokine profile seems to correlate well with the grade and severity of bladder cancer, Peripheral CD4+ T cells from bladder cancer patients have also been observed to

**Fully automated ELISA kits to detect IL-4, IL-6 & IL-10 Conc.**

The components and reagents of ELISA kits of komabiotech company that was used for deterging the level of IL-4, IL-6 and IL-10 in patients with bladder cancer and control group. As show in the table (1) below.

**Table (1): The components and reagents of Komabiotech ELIS kits**

components	Amount
Pre-coated 96 well ELISA microplate	1 plate
Biotinylated Affinity Purified Detection Antibody (lyophilized)	2 EA
Recombinant Standard Protein (lyophilized)	2 EA
Striptavidin-HRP Conjugate (0.6 ml)	1 EA
Assay Diluent (50 ml) : 1% BSA in PBS	1 EA
Assay Diluent G (10 ml) : N\A	N\A
TMB or pink-ONE Solution (10 ml)	1 EA
Stop Solution (10 ml)	1 EA
Wash Buffer Concentrate (20X, 50 ml) to make 1 liter	1 EA
Plate sealer	3 EA

**Statistical analysis**

Statistical analysis was carried out by using SPSS VER.23 two way T test (student’s T-test) and chi square to find out the statistical differences between all variables. probability less than 0.05 is significant (P<0.05).

**Results**

**Concentrations among studied groups in relation with gender**

Table (2) and figure (1) documented that the mean levels of interleukin IL-4 concentrations (pg\ml) among male and female of bladder cancer patients was higher than male and female of control group with (85.01±16.74), (9.87±6.16), (114.61± 64.16) (4.06±2.65) respectively, statistically the differences was highly significant with (P-value= 0.00, 0.008) respectively.

express increased IL-10 and other Th2 cytokines, along with decreased Th1 cytokines (Agarwal, *et al.*, 2006).

**Materials and methods**

**Sampling**

A Case-control study was conducted between October 2020 to July 2021 which carried for patients with bladder carcinoma according to minimum sampling size equation that depend on the disease ratio, the total number of bladder cancer patients involved in this study are (85) individual were taken from Basrah oncology center in Basrah province, the age of patients range from 30->60 years and (80) individual considered as control group after they were checked and confirmed to be free from any urological or any other clinical problems. during collection process data about each patient were reported in questionnaire paper for each one, which included age, gender, family history, smoking, alcohol drinking, occupation, residency and clinical findings of the disease. Bladder cancer patients selected at different tumor stage (T1-T4), also at different status (newly diagnosed, advance & recurrence).

**Table (2): Comparison of IL-4 concentrations (pg/ml) among studied groups in relation to gender.**

IL-4 (pg/ml)	Male	Female	t- test	p- value	C.S
	(Mean±SD.)	(Mean±SD.)			
Control	9.87±6.16	4.06±2.65	2	0.1	N.S
Patient	85.01±16.74	114.61± 94.16	2.2	0.4	N.S
t-test	23.7	3.1			
P-value	0	0.008			

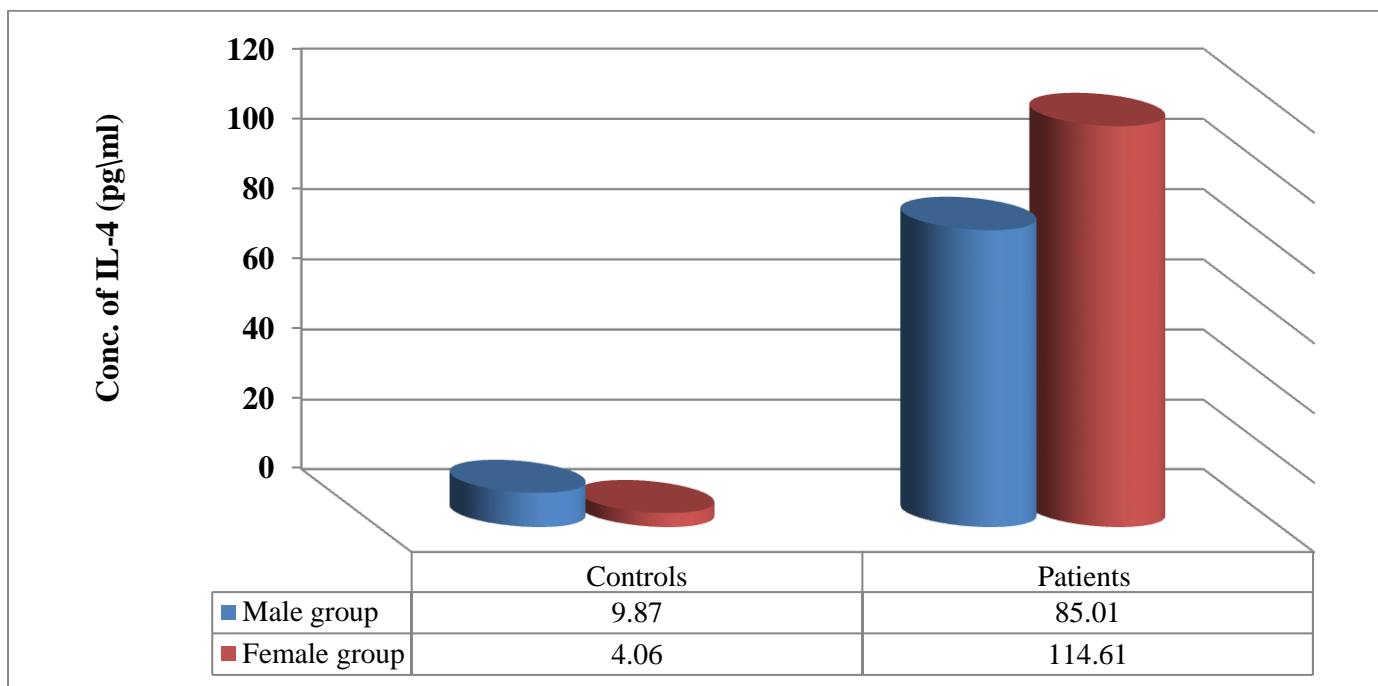


Figure (1): Comparison of IL-4 (pg/ml) among studied groups in relation to gender

**IL-4 Concentration in various age groups.**

Table (3) observed that the mean level of IL-4 (pg/ml) was higher among bladder cancer patients (50-59) years were (91.1 ±12.6), while less mean values of IL-4 (pg/ml) was lesser among bladder cancer patients under (40-49) years were (79.76± 15.57) ,statistically this differences was non- significant (P-value= 0.9).

**Table (3) IL-4 concentration among various age groups.**

Age groups (yrs)	Mean of Conc. (pg/ml)	NO.	Std. Deviation	Minimum	Maximum	P-value
(30-39)	83.37	4	25.91	56.3	106	0.9 (N.S)
40-49	79.76	6	15.57	56	99.6	
50-59	91.1	16	12.6	68	112.5	
>60	88.53	42	41.1	39.1	324.4	
Total	88.06	68	33.57	39.1	324.4	

**IL-4 Concentrations in comparison with tumor stages.**

Table (4) observed that the mean level of IL-4 concentrations (pg/ml) was higher among bladder cancer patients with tumor stage T3 were (106.05 ±63.52), while lowest mean values of IL-4 (pg/ml) was for tumor stage T1 were (79.98± 20.24), statistically the differences was non- significant (P-value= 0.13).

**Table (4) IL-4 concentration with tumor stage**

Stages	Mean	NO.	Std. Deviation	Minimum	Maximum	P-value
T1	79.98	26	20.24	39.1	109.7	0.13 (N.S)
T2	86.13	23	16.08	52.4	112.5	
T3	106.05	14	63.52	71.4	324.4	
T4	88.54	5	14.32	73.8	104.8	
Total	88.06	68	33.57	39.1	324.4	

**IL-4 Concentrations in comparison with case status**

Table (5) observed that the mean level of IL-4 concentrations (pg/ml) was higher among bladder cancer patients at advanced state were (94.19 ±51.01), while lowest mean values was recorded among newly diagnosed bladder cancer patients were (84.3± 18.01) ,statistically the differences was non- significant (P-value= 0.5).

**Table (5) IL-4 concentration with case status.**

Cases	Mean	NO	Std. Deviation	Minimum	Maximum	P-value
Advanced	94.19	25	51.01	39.1	324.4	0.5 (N.S)
Recurrence	84.75	18	14.93	56.3	104.8	
newly diagnosed	84.3	25	18.01	47.9	109.7	
Total	88.06	68	33.57	39.1	324.4	

**BCG treatment**

Table (6) document that the mean levels of IL-4 concentration was higher in bladder cancer patient whose not received BCG (91.05±37.36) versus (80.87±21.1) for those whose received BCG , the differences was non-significant (P-value= 0.25).

**Table (6) IL-4 concentration with BCG treatment.**

BCG	Mean	NO	Std. Deviation	Minimum	Maximum	P-value
Yes	80.87	20	21.1	39.1	109.7	0.25 (N.S)
No	91.05	48	37.36	50.2	324.4	
Total	88.06	68	33.57	39.1	324.4	

**Interleukin 6 (IL-6)**

**IL-6 Concentration among studied groups in relation with gender.**

Table (7) and figure (3-8) document that the mean levels of interleukin IL-6 concentrations (pg\ml) among male and female of bladder cancer patients was higher than male and female of control group with [(64.61±38.28) and (12.21±4.05)] and [(93.79±59.77) and (9.02±1.88)] for male and female respectively, statistically the differences was highly significant with (P-value= 0.00,0.09) respectively.

**Table (7): Comparison of IL-6 concentrations (pg/ml) among studied groups in relation to gender.**

IL-6 Conc. (pg/ml)	Male	Female	t- test	p- value	C.S

	(Mean±SD.)	(Mean±SD.)			
<b>Control</b>	12.21±4.05	9.02±1.88	1.69	0.1	**N.S
<b>Patient</b>	64.61±38.28	93.79±59.77	1.26	0.2	N.S
<b>t-test</b>	10.2	3.74			
<b>P-value</b>	0.00 (H.S)	0.009 (**H.S)			

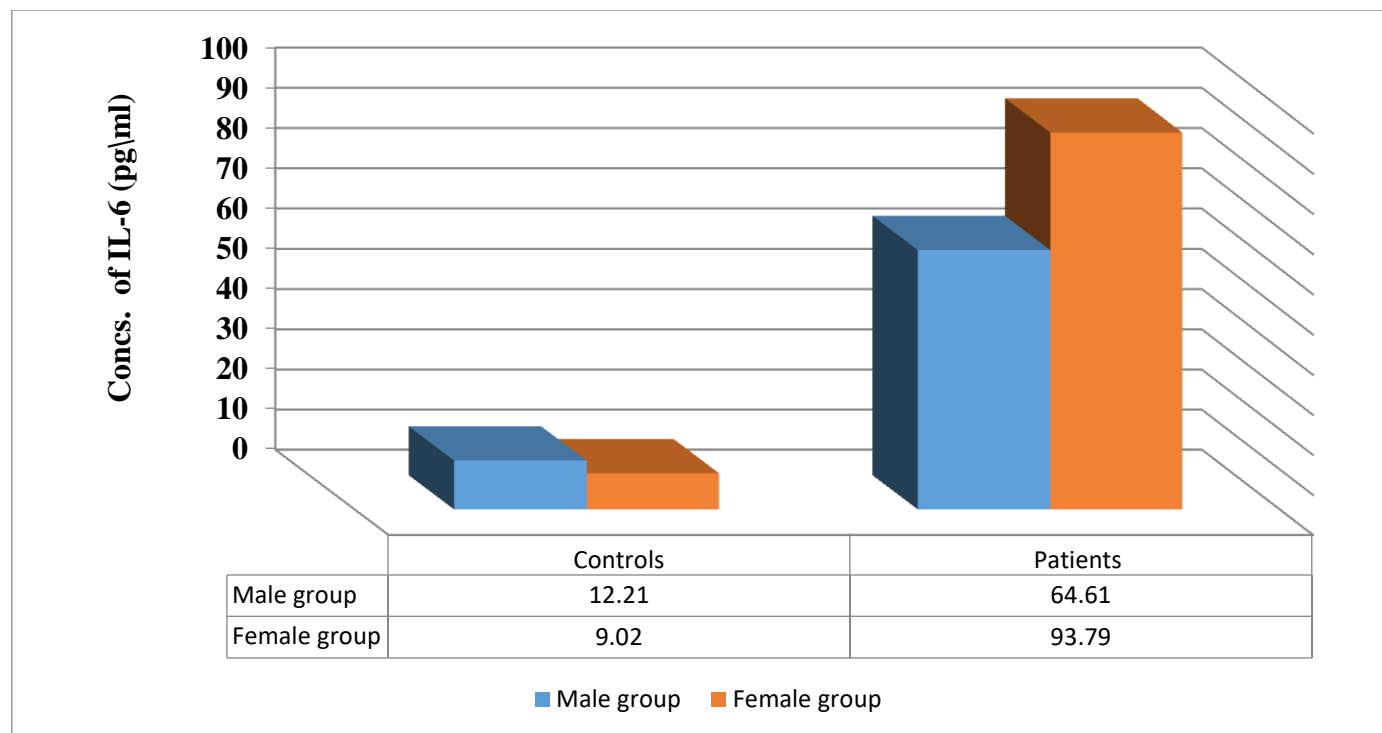


Figure (2): Comparison of IL-6 concentrations (pg/ml) among studied groups in relation to gender.

**IL-6 Concentration in various age groups.**

Table (8) observe that the mean level of IL-6 concentrations (pg/ml) was higher among bladder cancer patients >60 and (40-49) years were (70.14 ±46.8), (70.76 ±27.13) respectively , while lowest mean values of IL-6 (pg/ml) among bladder cancer patients under (30-39) years was (50.44± 16.75) ,statistically the differences was non- significant (P-value= 0.8).

**Table (8): IL-6 concentration among various age groups.**

Age groups (yrs)	Mean of Conc. (pg/ml)	NO	Std. Deviation	Minimum	Maximum	P-value
(30-39)	50.44	4	16.75	41.35	75.56	0.8 (N.S)
(40-49)	70.76	6	27.13	34.13	96.99	
(50-59)	64.09	16	35.29	33.65	146.9	
(>60)	70.14	42	46.8	34.13	281.67	
<b>Total</b>	67.61	68	41.38	33.65	281.67	

**Concentrations in comparison with tumor stages.**

Table (9) observe that the mean level of IL-6 concentrations (pg/ml) was higher among bladder cancer patients with tumor stage T3 were (81.12 ±76.41), while lowest mean values of IL-6 (pg/ml) was lesser among bladder cancer patients at tumor stage T2 was (52.39± 20.08) ,statistically the differences was non- significant (P-value= 0.16).

**Table (9): IL-6 concentration with tumor stage**

Stages	Mean of Conc. (pg/ml)	NO	Std. Deviation	Minimum	Maximum	P-value
T1	72.14	26	28.28	40.14	146.9	0.16 (N.S)
T2	52.39	23	20.08	33.65	105.62	
T3	81.12	14	76.41	34.13	281.67	
T4	76.29	5	17.06	51.68	90.24	
Total	67.61	68	41.38	33.65	281.67	

**IL-6 Concentrations in comparison with case status.**

Table (10) observe that the mean level of IL-6 (pg/ml) was higher among bladder cancer patients at recurrence state were (72.31 ±20.01), while lowest mean values of IL-6 (pg/ml) among newly diagnosed bladder cancer patients was (65.42± 29.9) ,statistically the differences was non- significant (P-value= 0.85).

**Table (10): IL-6 concentration with case status.**

IL-6	Mean of Conc. (pg/ml)	NO	Std. Deviation	Minimum	Maximum	P-value
Advanced	66.43	25	59.81	33.65	281.67	0.85 N.S
Recurrence	72.31	18	20.1	34.13	96.99	
newly diagnosed	65.42	25	29.9	39.9	146.9	
Total	67.61	68	41.38	33.65	281.67	

**BCG treatment.**

Table (11) document that the mean levels of IL-6 was higher in bladder cancer patient whose received BCG (78.53±24.158) versus (63.07±46.19) for those whose not received BCG , the differences was non-significant (P-value= 0.16).

**Table (11): IL-6 concentration with BCG treatment.**

BCG	Mean of Conc. (pg/ml)	NO	Std. Deviation	Minimum	Maximum	P-value
Yes	78.53	20	24.158	40.5	146.9	0.16
No	63.07	48	46.19	33.65	281.67	
Total	67.61	68	41.38	33.65	281.67	

**Interleukin 10 (IL-10)**

**IL-10 Concentrations among studied groups in relation to gender.**

Table (12) and figure (3) document that the mean levels of interleukin IL-10 concentration (pg/ml) among male and female of bladder cancer patients was higher than male and female of control group with [(57.83±23.95) & (12.61±3.39)] and [(81.81±32.98) & (11.54±0.36)] respectively, statistically this differences was highly significant with (P-value= 0.00,0.01) respectively.

**Table (12): Comparison of IL-10 concentration (pg/ml) among studied groups in relation to gender.**

IL-10(pg/ml)	Male	Female	t- test	p- value	Chi-Square
	(Mean±SD.)	(Mean±SD.)			
Control	12.61±3.39	11.54±0.36	0.8	0.4	**N.S
Patient	57.83±23.95	81.81±32.98	1.86	0.1	N.S
t-test	13.6	5.6			
P-value	0.00 (**H.S)	0.001 (**H.S)			

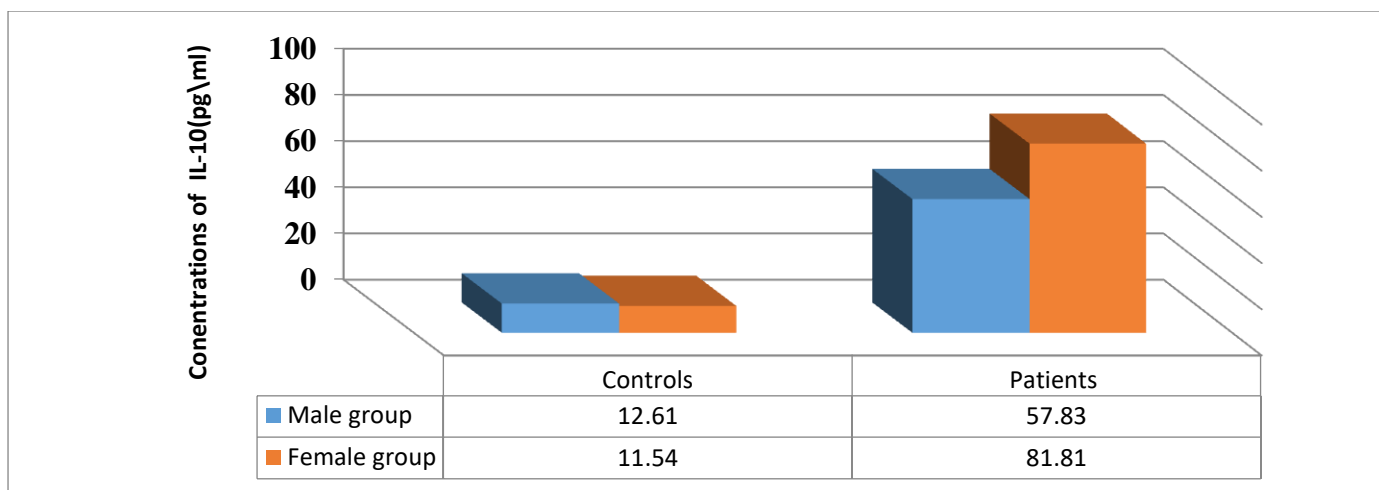


Figure (3): Comparison of IL-10 (pg/ml) among studied groups in relation to gender.

**IL-10 Concentrations in various age groups.**

Table (13) observe that the mean level of IL-10 concentration (pg/ml) was higher among bladder cancer patients (40-49) years were (63.05 ±31.96), while lowest mean values of IL-10 concentration (pg/ml) among bladder cancer patients under (30-39) years was (51.41± 10.71) ,statistically the differences was non- significant (P-value= 0.8).

**Table (13): IL-10 concentrations among various age groups.**

Age group (Yrs)	Mean of Conc. (pg/ml)	NO	Std. Deviation	Minimum	Maximum	P-value
(30-39)	51.41	4	10.71	39.53	65.23	0.8 (N.S)
(40-49)	63.05	6	31.96	25.39	118.9	
(50-59)	58.32	16	35.27	31.9	158.18	
(>60)	61.5	42	22.06	30.6	146.77	
<b>Total</b>	60.29	68	25.78	25.39	158.18	

**IL-10 Concentrations in comparison with tumor stages.**

Table (14) observe that the mean level of IL-10 concentration (pg/ml) was higher among bladder cancer patients with tumor stage T4 were (88.51 ±24.93), while lowest mean values of IL-10 (pg/ml) among bladder cancer patients at tumor stage T2 was (49.03± 14.25) ,statistically the differences was highly- significant (P-value= 0.007).

**Table (14): IL-10 concentrations in relation with tumor stage.**

Stages	Mean of Conc. (pg/ml)	NO	Std. Deviation	Minimum	Maximum	P-value
T1	65.66	26	27.07	30.6	158.18	0.007 (H.S)
T2	49.03	23	14.25	25.39	78.04	
T3	58.754	14	29.86	31.9	146.77	
T4	88.51	5	24.93	65.81	130.67	
<b>Total</b>	60.29	68	25.78	25.39	158.18	

**IL-10 Concentrations in comparison with case status.**

Table (15) observe that the mean level of IL-10 concentration (pg/ml) was higher among bladder cancer patients at recurrence state were (64.18 ±14.95), while lowest mean values of IL-10 (pg/ml) was lesser among newly diagnosed bladder cancer patients was (58.66± 27.53) ,statistically the differences was non- significant (P-value= 0.76).

**Table (15): IL-10 concentrations in relation with case status.**

IL-10	Mean of Conc. (pg/ml)	NO	Std. Deviation	Minimum	Maximum	P-value
Advanced	59.13	25	30.4	30.6	146.77	0.76 (N.S)
Recurrence	64.18	18	14.95	43.56	102.51	
newly diagnosed	58.66	25	27.53	25.39	158.18	
Total	60.29	68	25.78	25.39	158.18	

**BCG treatment.**

Table (16) document that the mean levels of IL-10 was higher in bladder cancer patient whose received BCG (72.68±27.32) versus (55.13±23.53) for those whose not received BCG , the differences was significant (P-value= 0.01).

**Table (16): IL-10 concentrations with BCG treatment.**

BCG	Mean of Conc. (pg/ml)	NO	Std. Deviation	Minimum	Maximum	P-value
Yes	72.68	20	27.32	30.6	158.18	0.01
No	55.13	48	23.53	25.39	146.77	
Total	60.29	68	25.78	25.39	158.18	

**Correlation result**

Table (17) observe that the mean level of concentrations for IL-4 (pg/ml), IL-6 (pg/ml) and IL-10 (pg/ml) were positive correlated with levels of Ages (years) as indicated by the score of (.014, 0.095, 0.041) respectively, statistically this correlation was non – significant (P-value=0.9, 0.4,0.7) respectively.

**Table (17): Correlation of Age (Years) with IL4 (pg/ml), IL6 (pg/ml) and IL10 (pg/ml) among bladder cancer patients.**

No	Interleukins	NO	r	p-value	Interpretation
1	IL-4	68	0.014	0.9 (N.S)	positive correlation
2	IL-6	68	0.095	0.4 (N.S)	positive correlation
3	IL-10	68	0.041	0.7 (N.S)	positive correlation

r, Pearson-correlation; N.S, non-significant

**Discussion**

**Interleukin 4**

Interleukin 4, a Th2-lymphocyte-derived immunoregulatory cytokine produced by activated CD4+ T cell, mast cell, basophils and eosinophil, IL-4 is the central differentiation factor driving Th2 development, inhibiting Th1 differentiation and eliminating extracellular pathogens. consequently, IL-4 plays an important role in surveillance and elimination of transformed cells. Various epidemiologic studies have examined the association between IL-4 and cancer risk so IL-4 and IL-4R play essentials roles in the development of a many carcinomas (Luo *et al.*, 2015), neutralizing of endogenous interleukin 4 lead to reduced tumor growth ( Li, *et al.*, 2008).

Numerous studies demonstrated that Th2 cytokines included IL-4 increased in urothelial carcinoma as mentioned in (Satyam, *et al.*, 2011) which determined the concentration of Th2 cytokines: IL-4 and IL-5 were significantly higher (P < 0.001) in patients than in controls. In the current study, results documented that the mean levels of interleukin IL-4 (pg/ml) among male and female of urothelial carcinoma patients was higher than male and female of control group with [(85.01±16.74) and (9.87±6.16)] and [(114.61± 64.16) and (4.06±2.65)] respectively, statistically the differences was highly significant with (P-value= 0.00, 0.08) respectively and confirm the results reported by the previous study (Satyam, *et al.*, 2011) that found significant increase of IL-4 (P < 0.001) in patients than in controls and the levels of IL-4 cytokine were



correlated with the grade and severity of the disease.

Aging is accompanied by many alterations in immune response due to the most consistent and significant changes occur in the T cell compartment, since cytokines are the core of immune cell communications, age related changes in cytokine production may contribute to these alterations (Bernstein & Murasko, 1998). In the current study we observed that the mean level of IL-4 was slightly higher among bladder cancer patients (50-59) years was (91.1 ±12.6 pg/ml), while slightly less mean values of IL-4 among bladder cancer patients under (40-49), (30-39) and (>60) years age groups were (79.76± 15.57 pg/ml), (83.37±25.91 pg/ml) and (88.53±41.1 pg/ml) respectively, statistically the differences was non-significant. there is no previous study explain the effect of aging on IL-4 serum level on patient with transitional cell carcinoma except (Bernstein & Murasko, 1998) study which done on murine and suggest that age associated TH2 cytokines included IL-4 production are not consistent and correlated with our study.

IL-4 consider as a cytokine that promotes tumor metastasis, The close correlation between interleukin-4 (IL-4) and tumor progression has been observed in plenty of studies, immune cells of cancer patients have increased activity to produce IL-4, and increased IL-4R expression on tumor cells indicates that IL-4 may play a critical role in tumor progression (Li, *et al.*, 2008). In the present study, observed that the mean level of IL-4 (pg/ml) was higher among bladder cancer patients with tumor stage T3 were (106.05 ±63.52), while slightly less mean values of IL-4 (pg/ml) among bladder cancer patients at tumor stage T1, T2 and T4 were (79.98± 20.24), (86.13±16.08) and (88.54±14.32) respectively, statistically the differences was non-significant, from these results we concluded that IL-4 serum level slightly elevated as the tumor progress from T1 to T2 and T3 but a little bit decreased at T4 may be attributed to manipulation with tumor microenvironment through multiple extirpations or treatment with biological therapy.

Bladder cancer cases in this study grouped into three divisions newly diagnosed, recurrence and advance cases and compared between cases status and IL-4 concentrations, mean level of IL-4 (pg/ml) was higher among bladder cancer patients at advanced state were (94.19 ±51.01), while lower mean values of IL-4 (pg/ml) among newly diagnosed and recurrence bladder cancer patients were (84.3± 18.01) and (84.75±14.93) respectively, statistically the differences was non-significant, tumor recurrence frequently occurs with low grade non-muscle invasive bladder cancer and almost large proportion newly diagnosed cases in the early stage of the disease so IL-4 concentration for these two groups almost equal, there are no previous studies compare between IL-4 serum level and case status.

Bacille Calmette-Guerin (BCG) is currently used to treat superficial bladder cancer, but despite its recognized effectiveness in preventing recurrence and progression, the immune mechanism behind its anti-tumor activity remains to be delineated (Magno, *et al.*, 2002). The present study documented that the mean levels of IL-4 was higher in

bladder cancer patient whose not received BCG (91.05±37.36) versus (80.87±21.1) for those whose received BCG, the differences was non-significant (P-value= 0.25), this results correspond and agree with previous study suggest that a prolonged increase in the plasma levels of IL-2, but not IL-1β, IL-4, IL-10, IL-2R or TNF-α occurred in patients affected by bladder cancer following effective BCG treatment (Magno, *et al.*, 2002).

### Interleukin 6

Interleukin 6 (IL-6) is one of the main cytokines in the tumor microenvironment. It is an important factor that is found at high concentrations and is deregulated in cancer. Its overexpression has been reported in almost all types of tumors. The strong association between inflammation and cancer is reflected in the high levels of IL-6 in the tumor microenvironment, which regulates all the signs of cancer and a variety of signaling pathways (including apoptosis, Survival, proliferation, angiogenesis, invasion and metastasis, and most importantly metabolism. In addition, IL-6 protects cancer cells from treatment-induced DNA damage, oxidative stress, and apoptosis by promoting the repair and induction of anti-signal (antioxidant and anti-apoptotic/pro-survival) pathways. Therefore, blocking IL-6 or inhibiting its related signals alone or in combination with conventional anti-cancer therapies may be a potential treatment method (Kumari, *et al.*, 2016).

There are plenty of studies suggest that IL-6 level increased among bladder cancer cases (Leibovici, *et al.*, 2005; Esuvaranathan, *et al.*, 2005 and Yeh, *et al.*, 2015). The present investigation documented that the mean levels of interleukin IL-6 (pg/ml) among male and female of bladder cancer patients was higher than male and female of control group with (64.61±38.28), (12.21±4.05), (93.79±59.77) (9.02±1.88) respectively, statistically the differences was highly significant with (P-value= 0.00,0.09) respectively, these result correlate the previous studies mention above (Esuvaranathan study conclude that, there was increase in IL-6 level in patients with UBC during BCG therapy p < 0.01). also, IL-6 level among female was much higher than male patients and there were no previous studies done before to confirm this result.

Aging is accompanied by many alterations in immune response due to the most consistent and significant changes occur in the T cell compartment, Overall, in the human system, the data on age-related IL-6 increases are not convincing, Elevated IL-6 levels have been clearly demonstrated in disease states related to aging (Bernstein & Murasko, 1998). In the current study, the mean level of IL-6 (pg/ml) was higher among bladder cancer patients >60 and (40-49) years were (70.14 ±46.8), (70.76 ±27.13) respectively, while slightly less mean values of IL-6 (pg/ml) among bladder cancer patients under (30-39) years were (50.44±16.75), statistically the differences was non-significant. there were no previous studies done on bladder cancer patients with aging effect on IL-6 level to be compare with it.

Interleukin 6 serum level correlated with higher clinical

stage of bladder cancer T1-T4 (Chen, *et al.*, 2013). In the present study we observed that the mean level of IL-6 (pg/ml) was higher among bladder cancer patients with tumor stage T3 were (81.12 ±76.41), while lowest mean values of IL-6 (pg/ml) seen among bladder cancer patients at tumor stage T2 were (52.39± 20.08), statistically this differences was non-significant (P-value= 0.16). the current result do not correspond with (Chen, *et al.*, 2013) which conducted on muscle invasive cases and measure IL-6 level in urine spicements, and (Cardillo, *et al.*, 2000) studies, we note that IL-6 serum level among different stages are somewhat close to each other except T2 stage which had the lowest level, that disparity of the results could be due to different types of treatment with biological therapy or chemoradiotherapy and manipulation with tumor microenvironment (Fisher, *et al.*, 2014).

The cases groups (newly diagnosed, recurrence and advance) that were classified in this study, the mean level of IL-6 (pg/ml) was higher among bladder cancer patients at recurrence state were (72.31 ±20.01), while slightly lesser mean values of IL-6 (pg/ml) among newly diagnosed and advance bladder cancer patients were (65.42± 29.9) and (66.43±59.81) respectively, statistically the differences was non-significant. IL-6 was significantly correlated with higher clinical stage, higher recurrence rate after therapeutic treatment, and diminished survival rate, according to reports IL-6 play multiple functions in angiogenesis and vascular modeling and increase angiogenesis by transcribing VEGF and MMP-9 in STAT3 dependent manner (Chen, *et al.*, 2013), so it's expected thing to increase in recurrence cases due to repeated growth of the tumor and also in advanced cases with increased tumor volume, these results correspond with previous study performed on urine sample and found elevated level of IL-6 in patients with locally advanced bladder TCC (transitional cell carcinoma) compared to patients with NMIBC (non-muscle invasive bladder cancer). Therefore, IL-6 expression might be related to a more malignant phenotype (Chen, *et al.*, 2013).

To determine whether BCG therapy could up regulate the cytokine (IL-6) production in human transitional cell carcinomas (TCC) this study measured the concentration of IL-6 for all patients to compare between patients who taken BCG therapy and those who did not taken it, and we found that the mean levels of IL-6 was higher in bladder cancer patient whose received BCG (78.53±24.158) versus (63.07±46.19) for those whose not received BCG, the differences was non-significant, the elevated level of interleukin 6 during BCG treatment may be caused by urothelial cells of the bladder and leukocytes the higher levels seen in non-responders may be due to higher grade or persistent tumors. The current study result agree with numerous studies also found elevated level of IL-6 in patients taken BCG (Esuvaranathan, *et al.*, 2005).

### Interleukin 10

IL-10 is essential factor for Th2 differentiation and has a major role as a suppressor of immune and inflammatory

responses, suppressing Th1 immune response at the tumor site and inducing a loss of tumor growth control, (Cardillo, *et al.*, 2000), this study documented that the mean levels of interleukin IL-10 (pg/ml) among male and female of bladder cancer patients was higher than male and female of control group with (57.83±23.95), (12.61±3.39), (81.81±32.98) (11.54±0.36) respectively, statistically the differences was highly significant with (P-value= 0.00,0.01) respectively, study results confirm the previous studies IL-10 increase among TCC patients and (Agarwal, *et al.*, 2006) results which found the mean levels of IL-6 and IL-10 in patients were significantly higher as compared to healthy controls, also the current result indicate that IL-10 level in female were higher than males, there were no previous studies compared IL-10 level among both gender.

Age-associated changes in cytokine production may contribute to T cell compartments alterations due to the major role of cytokines between cells communications (Bernstei & Murasko, 1998). The current study as documented in the table (3-19) indicate that the mean level of IL-10 (pg/ml) was higher among bladder cancer patients (40-49) years were (63.05 ±31.96), while less mean values of IL-6 (pg/ml) was lesser among bladder cancer patients under (30-39), (50-59) and (>60) years were (51.41± 10.71), (58.32±35.27) and (61.5±22.06), statistically the differences was non-significant (P-value= 0.8), there was no correlation between age and IL-10 level correspond with study result of Bernstei & Murasko (1998) which suggested that age-associated changes in cytokine production are not consistent because an age-associated decline in IL-10 production in response to specific stimuli is subtle and may be unmasked only by in vivo immune challenges, such as illness or vaccination.

Also we observed that the mean level of IL-10 (pg/ml) was higher among bladder cancer patients with tumor stage T4 were (88.51 ±24.93), while mean values of IL-10 (pg/ml) was lower among bladder cancer patients at tumor stage T1, T2 and T3 were (65.66±27.07), (49.03± 14.25) and (58.754±29.86) respectively, statistically the differences was highly-significant (P-value= 0.007), Interleukin 6 serum level correlated with higher clinical stage of bladder cancer T1-T4, the current study correlated with (Chen *et al.*, 2013) for patients at T4 and T1 stages but not with those of T2 and T3 where was the level of IL-4 decreased that disparity of the results could be due to different types of treatment with biological therapy or chemoradiotherapy and manipulation with tumor microenvironment.

In the three cases groups (newly diagnosed, recurrence and advance) we observed that the mean level of IL-10 (pg/ml) was higher among bladder cancer patients at recurrence state were (64.18 ±14.95), while less mean values of IL-10 (pg/ml) was lowest among newly diagnosed and advance bladder cancer patients were (58.66± 27.53) and (59.13±30.4) respectively, statistically the differences was non-significant (P-value= 0.76). IL-10 was significantly correlated with higher clinical stage, higher recurrence rate after therapeutic treatment, so it's expected thing to increase in recurrence cases due to repeated growth of the tumor and also

in advanced cases with increased tumor metastasis, these results correspond with previous study which suggested that no statistically significant difference was observed in the cytokine expression (IL-10) between patients showing recurrence and non-recurrence (Agarwal, *et al.*, 2006).

BCG is considered an important coadjuvant in the treatment of superficial bladder cancer, BCG instillation has the main goal of decreasing bladder cancer recurrences and preventing tumor progression, effective immunotherapy of bladder cancer by using Bacillus Calmette-Guerin (BCG) requires proper induction of Th1 immunity. Interleukin 10 (IL-10) down-regulates Th1 immune response and is related to the failure of BCG treatment. wherefore evaluated BCG plus IL-10 blocking antibodies this combination therapy induces enhanced Th1 immune response and anti-bladder cancer immunity (Luo, *et al.*, 2012). In the current study we compared IL-10 concentration between patient taken BCG and those who did not taken it which suggested that mean levels of IL-10 was higher in bladder cancer patient whose received BCG (72.68±27.32) versus (55.13±23.53) for those whose not received BCG, the differences was significant (P-value=0.01), these result correspond with (Chade, *et al.*, 2008) that found significant increase in IL-10 when compared with the control group (p<0.01).

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