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Urinary IL-8 and BLCA-4 in detection of bladder cancer and their clinical significant

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Abstract:

Bladder cancer is a highly prevalent human disease world wide This study of bladder cancer was conducted to assess the significance of level difference in some cancer biomarkers in bladder cancer patients, like IL-8 which is an angiogenic factor associated with inflammation and carcinogenesis and Bladder cancer-associated protein (BLCA4) that is a specific nuclear matrix protein (transcription factor) found in bladder cancer functioning in the regulation of the gene expression related to cell growth. IL8and BLCA4 evaluated in urine of 48 bladder cancer patients and 40 healthy controls by ELISA test, result indicated that urine IL8 and BLCA4 shows high specificity in diagnosis bladder cancer patients, The correlation between IL-8 with BLCA4 showed a significant association in patients with recurrent bladder cancer, urine IL8 and BLCA4 have a role in discrimination between (newly diagnosed vs recurrence, low grade vs high grade, muscle invasion vs non muscle invasion),but un related to some risk factors like smoking ,Schistosomiasis,Urinary tract infection,stones and family history of cance.

Keywords: *IL-8, BLCA4,Bladder cancer,Angiogenesis , Nuclear matrix protein, Elisa and carcinogenesis*

Introduction:

Global annual incidence rate of Bladder cancer is 350000 [1]. In the U.S.A an estimated new cancer cases and deaths from it in 2015 are 74,000 and 16,000 respectively [2]. It's the 5th most common cancer in Europe[3], with highest incidence rates in Southern Europe and lowest in Western Africa[4]. In Iraq, according to WHO data published in April 2011, Bladder cancer deaths reached 977 or 0.52% of total deaths, with the age adjusted death rate is 7.55 per 100,000 of population ranks Iraq 3rd in the world [5].

IL-8It is also called CXCL8, which is a soluble protein of 8-kDa molecule [6], it belongs to a superfamily of chemically related chemokines that stimulate neutrophil chemotaxis and degranulation, its levels is low in healthy tissues [7], however, it is rapidly generated in the presence of different stimuli including inflammatory signals (e.g., tumor necrosis factor α and IL-1β) , chemical and environmental stresses (e.g., exposure to chemotherapy and hypoxia), and steroid hormones (e.g., androgens, estrogens, and dexamethasone),[6]. It is secreted by a variety of normal and malignant epithelial cells of different types of cancer such as renal cell carcinoma[8], gastric carcinoma [9],and ovarian carcinoma [10]. Induction of IL-8 expression is mediated primarily by the transcription factor nuclear factor kappa B (NF-κB) [11]. However, the Src/signal transducer and activator of transcription 3 (Stat3) pathway may also promote IL-8 production independent of NF-κB [12]. So Interlukin-8 has potential role in malignant transformation of urothelial cells [13]. Sheryka etal.,2003 and Sagnak etal.,2009 demonstrated an elevated urinary protein levels of IL-8 in urothelial cell carcinoma associated with increased stage of disease and disease recurrence respectively[14,15], while similar elevation were found during lack of efficacy of intravesical therapies such as mitomycin C [16] . So, urinary IL-8 was the most accurate single biomarker when monitored by individual ELISA with a sensitivity of 87%, specificity of 86 %[17].

Getzenberg etal.,1996 identified six bladder-specific nuclear structure proteins (BLCA-1 to 6) that were expressed exclusively by bladder cancer cells, with changes in nuclear

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structure can affect gene expression[18], so playing an important role in the carcinogenesis process [19]. Therefore, nuclear morphology is deeply influenced by NMPs, based on these findings, NMPs are a potential cancer markers, moreover as NMPs are released into urine and serum, so suggested their roles in cancer diagnosis [20]. BLCA4 up-regulated of genes related to enhance tumorigenesis and tumor invasiveness, including the cyclins, interleukin-8 (IL-8), thrombomodulin (TM), and interleukin-1alpha (IL-1α) [21].

BLCA-4 can be used as bladder cancer urinary markers to identify individuals in the early stages of bladder cancer, thus, affecting the therapeutic approach and prognosis of patients. Also, the absence of high BLCA-4 levels in patients with various benign urologic disorders, such as urinary tract infection, catheterization, or cystitis will further strengthen the potential of this factor [20]. BLCA-4 was significantly higher in the bladder cancer group with no relation to age, gender, growth pattern, grade or stage of the disease and it yields a sensitivity of 97.37% and specificity of 100%, but muscle invasiveness was related to a higher BLCA-4 level, as well as 41.67% of adjacent normal tissue we found positive BLCA-4 expression [22], so the aim of this study is to evaluate the significances of some biomarkers of bladder cancer such as IL-8and BLCA-4 in urine (as non- invasive procedure),and Compare these biomarkers in patients at different cancer grade, invasion and relation with risk factors, then evaluate their effect on the prognosis of the illness.

Material and Methods:

This study consisted of 88 individuals, of which urine sample and tissue biopsy were collected from 48 bladder cancer patients (42men and 6 women with average age 63± 9.3) fromAl- Yarmouk and Baghdad Teaching Hospital over the period of study from June 2013 to April 2014. Ethical permission to conduct the research was obtained from these hospitals and from all patients included in this study. As well as normal urine sample were collected from 40 healthy volunteers (28men and 12women with average age 51±13.3), From each patient, a full medical history for diseases and previous laboratory finding was obtained, besides a cystoscopic examination by which transurethral resection (TUR) biopsies were taken from the apparent lesion, processed by standard oncological procedures, by(neutral buffered formalin10%, dehydrated through a graded series of ethanols, cleared in xylenes then embedded in paraffin and were stained with routine hematoxylin and eosin stains, following the procedure described in the texts of [23]. Hematoxylin and eosin section were examined for histological grading and muscle invasion examination. The tumor grade and muscle invasion was defined by a specialist pathologist according to(WHO/ISUP) and American Joint Committee on Cancer (AJCC, 7th ed.,2010).

• Urine collection and ELISA.

After collection of freshly voided urine samples in clean sterile

Container in cool box. Sample (50) ml were centrifuged at 3000 rpm for 10 minutes at 4°C and the supernatant was aliquoted, stored at −80°C in deepfreez until analyzed. Before analysis, samples were slowly thawed and Centrifuge again before assaying to remove any additional precipitates that may appear after storage. Urinary level for IL8(abcam), BLCA4 (cusabio) were quantitatively determined and done for collected samples accordingly . Enzyme-Linked Immunosorbent Assay (ELISA) for IL8 and BLCA4 performed for patients and healthy urine samples by commercially available ELISA kits. ,The lower level of sensitivity for the IL8 is 1pg/ml and The lower level of sensitivity for the-BLCA4 is 0.156 ng/ml The assay were read in a microplate reader and method is a solid phase sandwich ELISA .

Statistical Analyses:

Statistical analysis and reporting of obtained data were carried out by using the computerized database structure; Statistical Package for Social Sciences (SPSS V. 20) computer software was used for this purpose; sometimes, Curve Expert 1.4 (A comprehensive curve fitting system for windows) were used to plot curves as far it recommended by the diagnostic kits manufactures.

Frequency distributions were done for the study variables. Data were reported and presented as mean ± SD and/or (95% confidence interval) for the normally distributed variables.

The statistical significance of difference between mean of a normally distributed quantitative (continues) variables of two groups was assessed using the independent samples Student's t-test, and the Analysis of variance (ANOVA) were used to compare continuous variables between more than two groups, Statistical tests were approved by assuming a null hypothesis of no difference between mean of variables, a P values ≤ 0.05 was considered statistically significant.

The statistical significance (direction and strength) of linear correlation between two quantitative (parametric) variables was measured by using Pearson correlation. Coefficient Receiver Operating Characteristic (ROC) analysis and Curves were used to obtain and plot the area under curve (AUC) values for assessment of predictive accuracy of tests (sensitivity and specificity); also ROC analysis was used for evaluation and comparing data that produce the predictions and deciding about the cut-off points (threshold) that distinguish between positive and negative results.

Results:

• Assessment of IL-8

• Urine IL-8 level

Forty eight urine samples of bladder cancer patients and forty urine samples of healthy controls were analyzed by sandwiches ELISA test for assessment of urine IL8 levels. The mean ± SD (pg/ml) of urine IL8 level for patients was (310.8 ± 160.2) while the healthy controls was (62.04±37.66), with a statistical significant difference (P< 0.001) as shown in (Table1)below.

Table 1: Mean, SE and mode of IL-8 in bladder cancer patients and healthy controls.

IL-8 (pg/ml)	Bladder Cancer	Healthy volunteers
.No	48	40
Mean	310.83	62.04
Standard Deviation (SD)	160.20	37.66
Standard error of Mean (SE)	23.12	5.95
Mode	600	100.89
T test P value	12.2 0.001>	

• level of IL-8 in bladder cancer groups:

Then when we compare between healthy control, newly diagnosed and recurrent patients on regard to IL-8 level in urine, it showed that the mean ± SD was 62.04± 37.66, 265.95±139.09 and 359.6± 170.1 respectively, with a statistical analysis of IL-8mean level between groups of patients by using ANOVA test, showed that there was a significant difference between and within groups(F test 73.117, p< 0.001), as seen in (Table 2).The distribution of bladder cancer cases and healthy control according to the median IL-8 level (pg/ml) is shown in the (Figure 1).

Table 2: Mean, SD, SE and mode of IL-8 in healthy control, newly diagnosed and recurrent bladder cancer cases.

IL-8 pg/ml	Healthy controls	Newly diagnosed	Recurrence
No.	40	25	23
Mean	62.04	265.95	359.6
Standard Deviation (SD)	37.66	139.09	170.1
Standard error of Mean (SE)	5.95	27.8	35.4
Mode	100.89	170.5	600
Minimum	11.91 90		90.1
Maximum	109.34	600	600
Percentile 05th	13.61	116.1	120.9
25th	21.53	170.5	209.6
50th (Median)	50.12	190.8	331.2
75th	102.61	382.5	501.7
95th	106.52	493.6	600
99th	108.56	577.9	600
F test P value	73.117 0.001>		

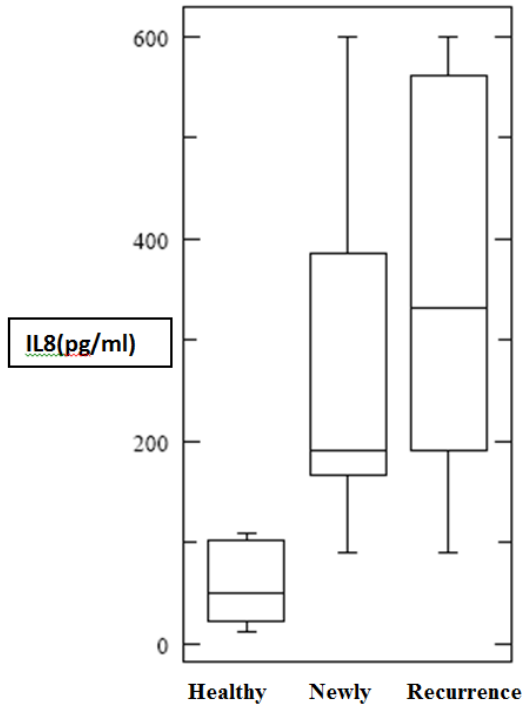

Figure1: Distribution of median IL-8 Level in healthy, newly and recurrent bladder cancer cases.

According to the age and gender, (Table 3, 4, 5 and 6) demonstrate, the mean ± SD (pg/ml) of urine IL-8 showed to be non-significant for patients and healthy subject.

Table 3: Mean, SD of IL-8 in different age groups of bladder cancer patients.

IL-8(pg/ml)	Age in years			
	<50	50-59	60-69	>70
No.	2(4.16%)	10(20.83%)	26(54.16%)	10(20.83%)
Mean	239.2	250.3	300.8	411.5
Standard Deviation	21008	130	150	183
F test	2.981			
P value	0.061			

Table 4: Mean, SD and SE of IL-8 in bladder cancer patients according to gender.

IL-8(pg/ml)	Gender	
	Male(42)	Female(6)
Mean	301.1	378.8
Standard Deviation	163.9	120.8
Standard error of Mean (SE)	25.29	49.32
Median	253.1	357.04
T test	1.11	
Pvalue	0.27	

Table 5: Mean ± SD of IL-8 in different age groups of healthy

IL-8(pg/ml)	Age in years			
	<50	50-59	60-69	>70
Mean ± SD	61.71 ± 34.3	63.40± 42.10	54.38± 42.29	73.07 ±43.32
F test	0.2425			
P value	0.87			

Table6: Mean ± SD of IL-8 in healthy according to gender.

IL-8(pg/ml)	Gender	
	Male(42)	Female(6)
Mean ± SD	61.46 ± 38.66	63.40 ± 36.84)
P value	0.881	
Sig.	p > 0.05	

• Urine IL-8 level in bladder cancer patients in relation to tumor grade:

Histological examination of 57 tissue samples of suspected bladder cancer patients, according to the (WHO/ISUP) classification system, showed that 30(52.63%) were low grade and27(47.36%) were high grade.

Urine samples were obtained from 48 out of these patients and analyzed by sandwiches ELISA test for assessment of urine IL-8 levels. In comparison between both groups, the mean urine level of IL-8 in low grade and high grade were (238.7 vs 403.5) with a highly significant increase in high grade(P = 0.0002), as shown in(Table 7 and Figure 2).

Table 7: Mean urine IL-8 level in bladder cancer patients in relation to tumor grade.

IL8 level	Low grade(27)	High grade(21)
Mean	238.7	403.5
Standard Deviation (SD)	143.9	131.5
Standard error of Mean (SE)	27.7	28.7
Mode	190.8	600
Minimum	90	206.8
Maximum	600	600
Median	170.5	399.09
T – test Pvalue	4.08 0.0002	

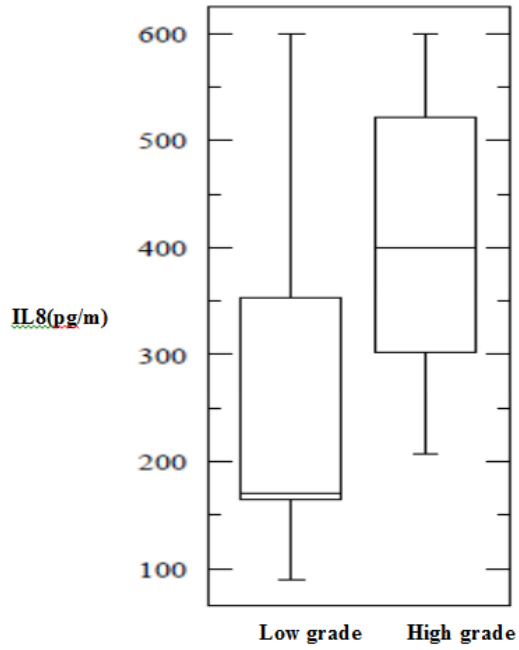

Figure 2: Median urine IL-8 level in bladder cancer patients in relation to tumor grade.

• Mean urine IL-8 level in bladder cancer patients in relation to tumor muscle invasion:

In comparing mean urine level of IL-8 of muscle invasion and non-muscle invasion tumor, it was (395vs246) respectively, with very high significant increase in muscle invasion (p =0.0009), as shown in(Table 8andFigure3).

Table 8: Mean urine IL-8 level in bladder cancer patients in relation to tumor stage.

IL-8 level	Muscle invasion (27)	Non -muscle invasion(21)
Mean	395	246
Standard Deviation (SD)	145	142
Standard error of Mean (SE)	31.5	27.4
Minimum	116.1	90
Maximum	600	600
Median	399	190.8
T –test P value	3.57 0.0009	

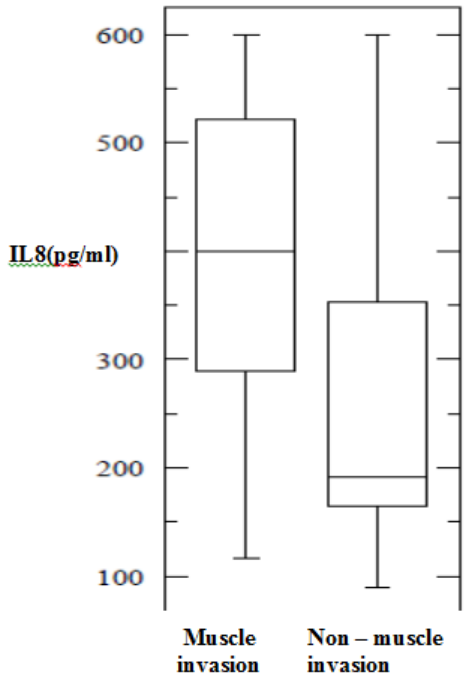

Figure3: Median urine IL-8 level in bladder cancer patients in relation to tumor stage.

• Urine level of IL-8 in relation to selected risk factors in bladder cancer cases:

The statistical analysis of urine IL-8 mean level in bladder cancer patients in correlation to selected risk factors by using student T-test is shown in (Table 9). With the mean level in smoking and non- smoking groups were (236.3vs202.4),whereas, 8out of 48 (16.66 %) of bladder cancer patients had a history of Schistosomiasis with mean level of 309.4 vs311.1when compared with non-schistosoma group, regarding presentation of patient with or without history of stones, UTI, and family history of cancer, IL8 urine level was(285.2vs 314.5), (306.3vs 317.6), (300.6vs312.8) respectively, as shown in table below, there were all with non-significant difference($p > 0.05$).

Table 9: Mean urine IL-8 level in bladder cancer patients in relation to risk factors.

IL-8 (pg/ml)	Smoking		*Schistosomiasis*		UTI		Stones		Family history	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
No. (%)	29 (60.41)	19 (39.58)	8 (16.66)	40 (83.33)	29 (60.41)	19 (39.58)	6 (12.5)	42 (87.5)	٧ (14.5)	٤١ (85.4)
Mean	236.3	202.4	309.4	311.1	306.3	317.6	285.2	314.5	300.6	312.8
SD	237.8	172.4	170.3	160.3	158.5	166.7	111.9	166.6	165.2	161.2
T-test	0.535		0.267		0.235		0.415		0.196	
P-value	0.60		0.98		0.82		0.68		0.85	

• Assessment of BLCA4:

• UrineBLCA4 level :

Forty eight urine samples of bladder cancer patients and forty samples of healthy controls were analyzed by sandwiches ELISA for BLCA4 levels. The mean ± SD (pg/ml) of urine BLCA4 level for patients was (1.378± 0.250) while for healthy controls was (1.025 ± 0.075), with statistically significant difference between them (P< 0.001), (Table10).

Table10: Mean urine BLCA4 level in patients and controls.

BLCA-4 (ng/ml)	Bladder Cancer	Healthy volunteers
No.	48	40
Mean	1.37	1.02
Standard Deviation (SD)	0.25	0.07
Standard error of Mean (SE)	0.03	0.01
Mode	1.21	1.01
Minimum	1.034	0.63
Maximum	1.92	1.11
Median	1.39	1.02
T test P value	8.57 0.0001	

• The BLCA4 level in bladder cancer groups.

Table11 shows the statistical analysis of BLCA4 mean level between groups of bladder cancer patients (newly diagnosed was 1.30± 0.22 and recurrent cases was1.45 ± 0.26) as well as healthy controls (1.02 ± 0.07), by using ANOVA test there were significant differences between and within groups. Also the distribution of healthy controls, newly diagnosed and recurrent bladder cancer patients according to the BLCA4 level (ng/ml) is shown in (Figure 4).

Table 11: Mean, SD ,SE and the mode of urine BLCA 4 in healthy, newly diagnosed and recurrent bladder cancer cases.

BLCA-4 ng/ml	Healthy controls	Newly diagnosed	Recurrent cases
Mean	1.02	1.30	1.45
Standard Deviation (SD)	0.067	0.22	0.26
Standard error of Mean (SE)	0.01	0.04	0.05
Mode	1.01	1.53	1.48
Minimum	0.63	1.034	1.10
Maximum	1.11	1.90	1.928
Percentile 05th	0.97	1.07	1.12
25th	1.005	1.15	1.20
50th (Median)	1.02 1.21		1.48
75th	1.06	1.51	1.52
95th	1.10	1.59	1.91
99th	1.11	1.85	1.92
F test Pvalue	43.67 0.000		

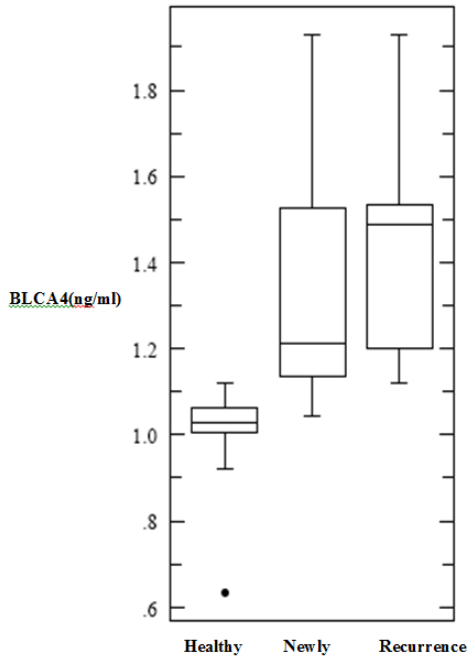

Figure 4: Distribution of median urine BLCA4 level through healthy,newly diagnosed andrecurrent bladder cancer cases.

For the age and sex, the mean ± SD (ng/ml) of urine BLCA4 in bladder cancer patients showed to be with significant difference for both age and sex(p= 0.0053 , p= 0.049)respectively as shown in (Table12 and 13), also ANOVA test shown significant difference in the mean ± SD (ng/ml) of urine BLCA4 for different age groups of healthy control, but non significant between male and female healthy group (Table14, 15) .

Table 12: Mean,SD,SE,and the mode of urine BLCA4 level in bladder cancer patients according to age.

BLCA-4 (ng/ml)	Age in years				F test Pvalue
	<50	50-59	60-69	>70	Ftest
No.(%)	(4.16)2	(20.83)10	(54.16)26	(20.83)10	5.929
Mean	1.36	1.25	1.34	1.58	
Standard Deviation	0.34	0.14	0.25	0.22	Pvalue 0.0053

Table13: Mean,SD,SE and mode of BLCA-4 in male and female bladder cancer patients.

BLCA-4 (ng/ml)	Gender	
	Male (42)	Female (6)
Mean	1.35	1.57
Standard Deviation	0.24	0.18
Standard error of Mean (SE)	0.038	0.07
Median	1.21	1.50
Ttest Pvalue	2.03 0.049	

Table 14: Mean ± SD of BLCA-4 in different age groups of healthy .

BLCA4 (ng/ml)	Age in years			
	<50	50-59	60-69	>70
Mean ± SD	0.04 ± 1.05	0.13 ± 0.98	0.01 1.004±	±0.03 1.00
F test P value	2.482 0.049			

Table15 : Mean ± SD of BLCA4 in healthy according to gender.

BLCA-4 (ng/ml)	Gender	
	Male	Female
Mean ± SD	0.08 ± 1.01	±0.04 1.03
P value	(P>0.05) 0.45	

• Urine BLCA4 level in bladder cancer patients in relation to tumor grade:

The mean urine level of BLCA-4 in low grade and high grade were (1.23 vs1.57) with a significant increase in high grade ($p < 0.001$) as shown in (Table 16).

Table 16: Mean urine BLCA4 level in bladder cancer patients in relation to tumor grade.

BLCA-4 (ng/ml)	Low grade	High grade
No.	27	21
Mean	1.23	1.57
Standard Deviation (SD)	0.207	0.158
Standard error of Mean (SE)	0.039	0.034
Mode	1.211	1.488
Minimum	1.034	1.433
Maximum	1.61	1.928
Median	1.165	1.60
T –test Pvalue	6.17 0.0001	

• Mean urine BLCA4 level in bladder cancer patients in relation to tumor muscle invasion:

In comparing the mean urine level of BLCA-4 between muscle invasion and non -muscle invasion, it was1.554 *vs*. 1.241 with very high significant increase in cases with muscle invasion ($p < 0.001$) (Table 17).

Table 17: Mean urine BLCA4 level in bladder cancer patients inrelation to tumor muscle invasion.

BLCA-4 level	Muscle invasion	Non- muscle invasion
No.	27	21
Mean	1.554	1.241
Standard Deviation (SD)	0.180	0.209
Standard error of Mean (SE)	0.039	0.04
Minimum	1.155	1.043
Maximum	1.928	1.61
Median	1.515	1.202
T –test P value	5.45 0.0001	

• Urine level of BLCA-4 in relation to selected risk factors in bladder carcinoma:

Statistical analysis of urine BLCA4 mean level in bladder cancer patients in relation to some risk factors is shown in (Table18), with non-significant differences between different groups in regard to a specific risk factor as smoking, history of *Schistosomiasis*, history of stones, UTI and family history of cancer.

Table18: Urine level of BLCA4 in relation to selected risk factors in bladder carcinoma

BLCA-4 (ng/ml)	Smoking		*Schistosomiasis*		UTI		Stones		Family history	
	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
No. (%)	29 60.41	19 39.58	8 16.66	40 83.33	29 60.41	19 39.58	6 12.5	42 87.5	7 14.5	41 85.4.33
Mean	1.125	1.174	1.42	1.36	1.35	1.40	1.39	1.37	1.43	1.36
SD	0.178	0.178	0.28	0.24	0.3	0.25	0.24	0.25	0.311	0.24
T-test Pvalue	0.938 0.35		0.532 0.60		0.642 0.52		0.172 0.86		0.655 0.52	

• Correlation between urine means level of IL-8 with BLCA4 of healthy subjects, newly and recurrent bladder cancer groups:

The correlation between urine IL-8 with BLCA4 mean level in bladder cancer groups (newly diagnosed and recurrent case) and control is shown in Table(19), of which the data shows a highly significant association (P= 0.000) between them in patients with recurrent bladder cancer, while there was no significant association in the other groups.

Table 19: The correlation between urine mean level of BLCA4 with IL-8 mean level in healthy subjects and bladder cancer groups.

Control		IL-8 pg/ml		
		Newly	Recurrence	
BLCA4 (ng/ml)	R	0.132	0.189	*0.728
	P	0.418	0.366	0.000

* Correlation is significant at the level of 0.01, R: (pearson correlation)

• Receiver Operating Characteristic (ROC) analysis:

This analysis were used to evaluate the performance of diagnostic tests and more generally for evaluating the accuracy of a statistical model that classifies subjects into one of two categories, diseased or not, with the area under the ROC curve (AUC) gives an idea about the usefulness of a tested parameter in differentiating between groups, the closer the area to one, the more useful it is in discrimination. So this ROC was used to determine the cut-off value for different parameters for diagnosis of bladder cancer as well as differentiating them from healthy controls by the best test which is IL-8 as highly significant (P =0.000), with Roc area 0.983 with high sensitivity and specificity. Also BLCA4 were highly significant(P 0.000), with ROC area 0.977 with high sensitivity and specificity, as shown in(Table 20, Figure5 and 6) .

Table 20: The ROC analysis for urine IL-8 and BLCA4 level in bladder cancer patients.

	AUC	SE	95%CI	P-value	Cut-off	Sensitivity (%)	Specificity (%)
IL-8 pg/ml	0.983	0.012	0.959-1.000	0.000	105.6	95	85
BLCA-4 ng/ml	0.977	0.013	0.952-1.000	0.000	1.076	93	85

AUC : Area under ROC curve , SE : standard error , CI: confidence interval

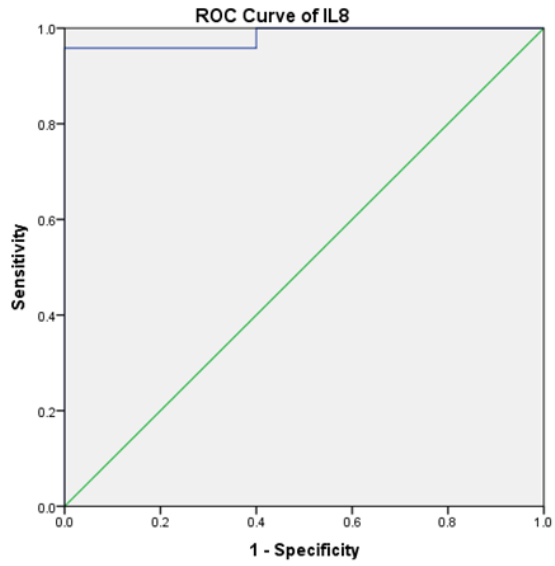

Figure 5: Receiver operating characteristic curve for detectionof bladder cancer patients by reference to the level of urine IL-8.

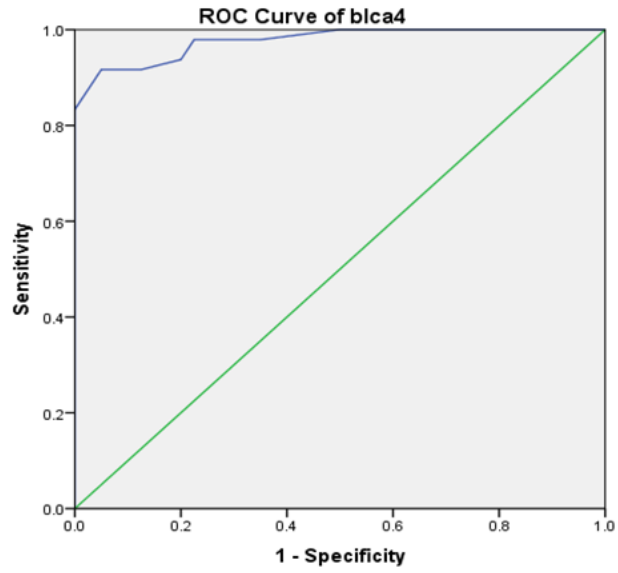

Figure 6: Area under the Receiver Operating Characteristic curve for detection of bladder cancer patients by reference to the level of urine BLCA4

Discussion:

Interlukine 8 has been evaluated in different types of human cancers, such as leukemia, ovarian cancer, breast cancer, prostate cancer and urinary system cancer. [24, 25, 26, 27, 28 , 29].

The present study, estimated urine IL-8 of 48 bladder cancer patients, 25(52%) newly diagnosed and 23(47%) recurrent cases, as well as,40 healthy control subject, the mean± SD of urine IL-8 for recurrent cases(359.6± 170.1) was significantly higher (P =value 0.001) than newly diagnosed (265.95±139.09), while the level was significantly getting down in healthy group(62.04 ± 37.66)when compared with newly and recurrent cases (P= 0.001). These result were in agreement with previous reports that documented an elevated urinary levels of IL-8 in patients with urothelial cell carcinoma[30,28,31,32], with significant increase in recurrent group than newly diagnosed[15].

On regard to tumor grade, this study shows a statistically significant increase in urine IL-8 level (P = 0.0002) in high grade TCC (403.5pg/ml) when compared with low grade tumor (238.7pg/ml). Moreover, patients with invasive bladder cancer had significantly higher level of IL-8(395pg/ml) than non –invasive patients(246pg/ml) with P value of 0.0009, such a finding were similar to[14,31]who indicated that elevated urinary levels of IL-8 are associated with increased stage of the cancer, while opposite to these were[32], of which they did not find a statistical difference between high and low grade IL-8 serum levels. So the data in present study were strongly support the suggestion that, IL-8 contribute to human cancer through two mechanisms, direct effect on tumor cell growth by regulated adhesion, migration and invasion of cancer cells and indirect effects through attracting host infiltration cells and angiogenesis [33], as well as its high level will result in increased tumorigenicity, progression then metastasis, with inhibition of tumor growth by anti-IL-8 antibodies via down-regulation of nuclear factor kappa-B [34]. So as IL-8 is cytokine, it was expected and noted to bleed into stroma (as urine) and that could be used as a non-invasive bladder cancer diagnostic signature as well as, it has utility in prognostic evaluation when monitored in solid tissue and perhaps in urine [29].

For discrimination between disease presence and absence, ROC analysis was used in several studies to identify the cut-off value of different cytokines [31],in this study, the urine level of IL-8 at cut-off value of (105.6pg/ml) in bladder cancer patients was highly significant (P= 0.000), with high sensitivity and specificity(95%,85%), when compared with healthy group which indicate that significant increase in cytokines concentration as an early sign for diagnosis of bladder cancer, that is to say that this is the best test for differentiation between healthy and bladder cancer patients, there was in agreement with resent study done by[17], of which they found that urinary IL-8 was the most accurate single biomarker when monitored by individual ELISA with a sensitivity of 87%, specificity of 86%, however Urquidi eta.,2012 reported 59%sensitivityand 97% specificity at median concentration of 128pg/ml of IL-8[30]. On regard to correlation of IL8 with risk factors of bladder cancer, this study show no significant correlation between IL-8with

these risk factors(smoking,Schistosomiasis,Urinary tract infection,stones and family history of cancer), may be some another tests or markers could explained some correlation with some of these risk factors

BLCA4This is a transcription factor functioning in the regulation of the gene expression in bladder cancer so it was regarded as a highly specific bladder cancer marker that expressed early in the development of the disease[35,21]. In this study we noted a highly significant increase ($P < 0.0001$) in its level in patients mean ± SD(1.378± 0.250) when compared with healthy subjects mean ± SD(1.025 ± 0.075) with the highest urine BLCA4 mean level from recurrent bladder cancer patients(1.45 ng/ml) with significant increase($P < 0.000$) than newly diagnosed group (1.30 ng/ml) with the lowest were in healthy groups(1.02ng/ml),this was comparable to [22, 20, 36] in considering Urinary BLCA-4 was significantly higher in the bladder cancer group than healthy.

Recently uBLCA-4 showed a potential utility as a urine-based bladder tumor marker [36], of which it affects the pathogenesis of bladder cancer through increasing of both, the levels of thrombomodulin (which maintains blood flow), as well as IL-1α and IL-8 (mediate cellular proliferation, invasion, and angiogenesis[21].

In high grade TCC mean level BLCA4 (1.57ng/ml)was statistical significant increase in comparison to low grade (1.23ng/ml)with ($p < 0.001$)and this was analogous to previous study, which points a role of BLCA-1 and BLCA-4 in identifying the early stages of bladder cancer [20]. Regarding muscle invasion, patients with invasive bladder cancer had significant higher level of BLCA4 (1.554ng/ml) than non –invasive one (1.241ng/ml) with P value 0.0001, this was similar to Feng etal.,2012 of which such finding were strongly support the suggestion that BLCA-4 enhance tumourigenesis and tumour invasiveness through its interaction with IL-1α, IL-8, VEGF and MMP-9 but, may not effect pro-angiogenic pathways in bladder cancer [37].

Sensitivity and specificity of urinary BLCA-4 by ELISA tests reached(93.8% and 85%) respectively, at cut-off value (1.076 ng/ml) and the area under the curve for the urine BLCA-4 tests was 0.977, however, Feng etal.,2011 revealed 97.3% sensitivity and100% specificity[22], and recently Xia etal.,2014 reported sensitivity and specificity of 85% and 97% respectively and the area under the curve for the urine BLCA-4 tests was 0.9806[36], from these finding besides an absence of high BLCA-4 levels in individuals without bladder cancer (included roughly benign genitourinary conditions)we can use urinary BLCA-4 for the screening of bladder cancer[35, 20].

When evaluating the level of urinary BLCA4 with risk factors, this study found a significant correlation between urine BLCA4 level and patients age and sex, however no statistical correlation was found in present study between BLCA4 with other risk factors which include: smoking ,Schistosomiasis , UTI, stones and family history. Different result was reported by Feng etal.,2011 which revealed no statistical correlation between BLCA4 with age and sex[22], but a correlation demonstrated between this factor with age and gender, as well as overexpression in invasive stage which seems to increase the growth rate in cells and also causes cells to express a more tumorigenic phenotype[38].

The correlation between different studied factors with each other (in different groups) showed a highly significant association between urine IL-8 with BLCA4 (P 0.000), in patients with recurrent bladder cancer, while there was no significant association in the other groups. These finding were similar to previous study that showed a positive correlations between BLCA-4 with IL-8 ($p=0.001$) with such a relation mainly obtained in cases of advanced and recurrent cases of cancer [37]. As well, present study were in agreement with[20] who found that an up-regulation of IL-8in overexpressing BLCA-4 cells that result in increasing tumor growth and metastasis.

The conclusion of this study is that IL-8 and BLCA-4 in urine measured by ELISA shows high specificity in bladder cancer patients than normal, with urine IL-8 and BLCA-4 have a role in discrimination between newly diagnosed and recurrent bladder cancer patients as well as low grade, non- muscle invasion and high grade, muscle invasion bladder cancer.

There is a significant correlation between IL-8 with BLCA-4 in recurrent bladder cancer group only, with marked increase in its concentration.

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دور IL-8, BLCA4 في الكشف عن سرطان المثانة واهميته السريرية

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الخلاصة:

سرطان المثانة من الامراض الواسعة الانتشار في العالم وقد اجريت هذه الدراسة لتقييم اهمية مستوى الفرق في بعض المؤشرات الحيوية للسرطان في مرضى سرطان المثانة مثل IL-8 الذي يعتبر عاملا مولدا للاوعية الدموية ومرتبطا بالالتهابات والسرطان وبروتين سرطان المثانة المصاحب (عامل النسخ(BLCA4 الذي يعمل على تنظيم التعبير الجيني المتعلق بنمو الخلايا، تم تقييم مستوى BLCA4 و IL-8 في بول 48 مريضا بسرطان المثانة و 40 من الاصحاء بواسطة اختبار الاليزا، وأشارت النتيجة أن IL8 و BLCA4 أظهرت خصوصية عالية في تمييزمرضى سرطان المثانة و وجود ارتباط كبيرة بين IL-8 مع BLCA4 في المرضى الذين يعانون سرطان المثانة المتكرر،كما ان IL-8 مع BLCA4 لهما دور في التمييزبين (المشخصين حديثا ومتكررين الاصابة، درجة وتصنيف الورم، لكن ليس لهما اي صلة مع بعض عوامل الخطر المصاحبة لسرطان المثانة مثل التدخين، البلهارزيا ،التهاب المسالك البولية ،الحصى والتاريخ العائلي للمرض).