

## Significance of Interleukin-8 in Cystitis and Schistosomal Chronic Cystitis

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### Abstract:

One of the important mediators of early inflammatory response in cystitis and an agent for attraction of neutrophils to the inflammation site is IL-8, in addition to its role in regulation angiogenesis. This study was carried out to assess the level of IL-8 biomarker in urine of cystitis patients (schistosomal chronic cystitis and non schistosomal cystitis).

Eighty eight Iraqi individual were divided to 50 cystitis patients and 38 healthy control, of whom urine sample and bladder biopsy were collected. Diagnosis of cystitis patients carried out by cystoscopy followed by histopathological examination and was classified to schistosomal chronic cystitis and non-schistosomal cystitis. Urine IL-8 level of studied groups was measured by ELISA test.

According to the result of ELISA technique, the mean urine concentration (pg/ml) of IL-8 increased significantly in cystitis patients 224.86 pg/ml than healthy controls 51.70 pg/ml. In regard to the age of cystitis patients urine level of this marker was significantly different ( $P < 0.05$ ), but there was not any significant increase in IL-8 urine level according to sex, while IL-8 was with significant increase in Schistosomal chronic cystitis than non-Schistosomal cystitis. Lastly urine IL-8 showed specificity in diagnosis of cystitis 91.39% and more sensitivity 96.34%.

Result of this study showed significant increase in urinary IL-8 concentration in cystitis patients, with significant correlation between IL-8 level with Schistsoma infection and age.

**Key words:** IL-8, Cystitis, ELISA, Schistsoma and Histopathology.

الأهمية السريرية للإنترلوكين-8 في التهاب المثانة المصاحب وغير المصاحب للبلهارزيا

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### الخلاصة:

الإنترلوكين-8 هو أحد العوامل المهمة في الاستجابة الالتهابية المبكرة من التهاب المثانة فضلاً عن دوره في جذب خلايا العدلة الى مواقع الالتهاب ودوره في تنظيم التكون الوعائي.

الهدف من هذه الدراسة هو تقييم مستوى المؤشر البايولوجي الانترلوكين-8 في بول مرضى التهاب المثانة البلهارزي المزمّن وغير البلهارزي.

تم تقسيم ثمانية وثمانين فرد عراقي الى قسمين، الأول مكون من (50) مريضاً بالتهاب المثانة و(38) شخصاً من الأصحاء. تم جمع عينات بول وخزغ من المثانة وتم تشخيص مرضى التهاب المثانة من خلال ناظورالمثانة وفحص الأنسجة ومن ثم تصنيف المرض الى التهاب المثانة البلهارزي المزمّن والتهاب المثانة غيرالبلهارزي، وقد تم قياس الانترلوكين-8 في بول المجموعات المدروسة من خلال تقنية الاليزا.

وفقاً لنتيجة تقنية الاليزا ظهر زيادة إحصائية في تركيز الانترلوكين-8 في بول مرضى التهاب المثانة عن الاصحاء. أما فيما يتعلق بعمر مرضى التهاب المثانة فقد كان هناك إختلافاً إحصائياً بمستوى الانترلوكين-8 بين الأعمار المختلفة، ولكن لم يكن هناك أي زيادة في تركيز الانترلوكين في البول حسب الجنس. كذلك لوحظ وجود اختلافات معنوية في تركيز الانترلوكين-8 في مرضى التهاب المثانة البلهارزي المزمّن والتهاب المثانة غير البلهارزي. وأخيراً أظهرالانترلوكين-8 دقة 91.39% وحساسية أكثر 96.34% لتشخيص المرض.

أظهرت نتائج هذه الدراسة وجود زيادة في تركيز الانترلوكين-8 في بول مرضى التهاب المثانة فضلاً عن وجود علاقة ذات دلالة إحصائية بين مستوى الانترلوكين-8 في الادرار والاصابة بالبلهارزيا والعمر.

## **Introduction:**

Interleukin IL-8 is a chemotactic factor secreted by activated leukocytes promotes migration of T lymphocytes and neutrophils to the infection region and degranulation [1]. Different stimuli are rapidly generated IL-8, as chemical and environmental stresses like (chemotherapy and hypoxia), steroid hormones (estrogens, dexamethasone and androgens) and inflammatory signals (Interleukin-1 $\beta$ , tumor necrosis factor) [2]. IL-8 interacts with specific G protein-coupled CXC chemokine receptors (CXCRs) [3] in high affinity. Activation of this CXCRs leads to signal transduction [3].

Through urinary bladder the urothelial cells express but normally do not secrete IL-8 [4]. Urothelial cells increase the release of IL-8 in response to urinary tract infection [5], so it has a role in chronic and acute inflammation and induces migration of PMN cells to the urine in addition to promotes angiogenesis through stimulating growth and endothelial cells survival [6]. Ko et al., 1993 noted a correlation between

urinary tract infection and UT cytokines [7]. However its levels are low in healthy tissues [1].

Systemic inflammation shown to be induced by chronic schistosomiasis [8], and Schistosomiasis not only associated with increased of activated CD8 and CD4 cells [9], but also the innate immune defense, IL-8, Toll Like Receptors can be modulated through chronic schistosomal infections [10].

## **Materials and Methods:**

This study was carried out at AL-Yarmouk and Baghdad Teaching Hospitals in Baghdad from August 2015 to January 2016. A prospective study included 50 cystitis patients (36 male, 14 female; 32 to 72 years old), specimens of urinary bladder biopsies were obtained by cystoscopy, blocks of tissue were prepared for microscopic examination by fixation in 10% neutral formalin and then dehydrated in ethanol alcohol, embedded into paraffin wax, cut at thickness of 3-4  $\mu$ m, and then stained with hematoxylin-eosin (H-E) for histopathological examination under a light

microscope, the cystitis cases had been included in this study and were divided to schistosomal chronic cystitis and non-schistosomal cystitis, also urine sample were taken from those 50 patients through catheterization or freshly voided midstream.

The control group who had normal urinalyses consisted of 38 individual (25 male, 13 female; 33 to 73 years old). The urine IL-8 level was determined by an enzyme linked immunosorbent assay (ELISA) with (ABCAM KIT), 10 ml of urine were centrifugated at 3000 rpm for 10 min. at 4°C, the supernatant was aspirated and stored in deep freeze at -80°C for immunological analysis. Specificity and sensitivity of marker were estimated.

### **Statistical Analysis**

The Statistical Analysis System-SAS (2012) program was used to study the effect of different factors. T-test and ANOVA (Analysis of variation) was used to compare between means in this study. Receiver Operating Characteristic (ROC) was used for calculation the sensitivity and specificity of the test.

### **Results:**

#### **Assessment of urine IL-8 in cystitis patients**

By using ELISA test urinary IL-8 levels (pg/ml) were significantly higher in cystitis patients in comparison with control (224.86 vs 51.70), (P=0.0001), (Table-1; Figure-1).

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#### **Interleukin-8 urine level in relation to gender**

According to gender there was non-significant increase (P=0.3296) in IL-8 urinary level in male (209.17 pg/ml) than female (265.19 pg/ml), (Table-2).

#### **Level of IL-8 in urine according to age**

The comparison between different age groups in the mean level of urine IL-8 by using ANOVA test shows that there was a significant difference between and within groups with P value of 0.0448, as seen in (Table-3).

#### **Assessment of urine IL-8 level in regard to schistosoma infection**

Out of 50 cystitis patients, only 8 were with schistosoma chronic cystitis according to histopathological examination of bladder biopsies, T-test showed significant increase in IL-8 concentration in relation to schistosoma infection (P<0.05), as noted in (Table-4; Figure-2). No correlation was found between urinary IL-8 levels (pg/ml) and gender (240.07vs367.78) in male and female cystitis respectively (Table-5). In regard to age groups level of urine IL-8 was with significant statistical difference (P<0.05), (Table-6).

#### **Specificity and sensitivity of IL-8**

Receiver Operating Characteristic (ROC) analysis was used for discrimination between presence of disease and absence and to determined specificity and sensitivity of study marker urine IL-8 which were 91.39% specificity and 96.34% sensitivity in cystitis patients with P-value of 0.0001(Table-7).

**Table-1: Compare between cystitis patients and healthy control in mean urine IL-8 level**

Results	Cystitis group	Healthy group
No.	50	38
Mean(pg/ml)	224.86	51.70
SD	105.90	38.14
SE	14.96	6.18
Minimum(pg/ml)	105.86	20.67
Maximum(pg/ml)	600.13	200.81
T-test	35.836 *	
P-value	0.0001	
*Highly significant		

**Table-2: Mean urinary IL-8 level in cystitis group according to gender**

Results	Sex	
	Male	Female
No. (%)	36 (72.00%)	14 (28.00%)
Mean(pg/ml)	209.17	265.19
SD	96.77	120.93
T-test	61.309 NS*	
P-value	0.3296	
*NS: Non-significant.		

**Table-3: Urine IL-8 means level in cystitis patients in relation to age**

Results	Age (year)				
	30-39	40-49	50-59	60-69	≥70
No. (%)	2 (4.00%)	11 (22.0%)	15 (30.0%)	18 (36.0%)	4 (8.0%)
Mean(pg/ml)	202.73	195.05	200.17	272.42	196.39
SD	127.58	68.23	63.10	145.13	54.80
LSD-value (F.)	53.298 *				
P-value	0.0448				
* (P<0.05) significant					

**Table-4: Urine level of IL-8 in Schistosoma chronic cystitis and non-schistosomal cystitis**

Results	Schistosoma chronic cystitis group	Non Schistosoma chronic cystitis group
No.	8	42
Mean(pg/ml)	303.93	209.80
SD	131.78	94.79
SE	46.59	14.62
Minimum(pg/ml)	196.93	105.86
Maximum(pg/ml)	600.13	196.93
T-test	78.367 *	
P-value	0.0196	
* (P<0.05) significant		

**Table-5: Effect of gender in mean level of urine IL-8 in Schistosoma chronic cystitis group**

Results	Sex	
	Male	Female
No. (%)	4(50.0%)	4(50.0%)
Mean(pg/ml)	240.07	367.78
SD	43.80	166.52
T-test	89.336 NS*	
P-value	0.2781	
* (P>0.05) non significant		

Table-6: Mean urine level of IL-8 in Schistosoma chronic cystitis patients in relation to age groups.

Results	Age (years)		
	40-49	50-59	≥60
No. (%)	2(25.0%)	3(37.5%)	3(37.5%)
Mean(pg/ml)	260.01	219.73	417.40
SD	75.47	19.90	160.20
LSD-value (F.)	86.631 *		
P-value	0.0217		
* (P<0.05)significant			

Table-7: Sensitivity and specificity of urine IL-8 in cystitis patients

	Groups	SE	95% CI	Sensitivity (%)	Specificity (%)	P-value
IL-8	Cystitis	0.013	0.95-1.00	96.34	91.39	0.0001
	Control	0.02	0.96-1.00	92.53	86.45	

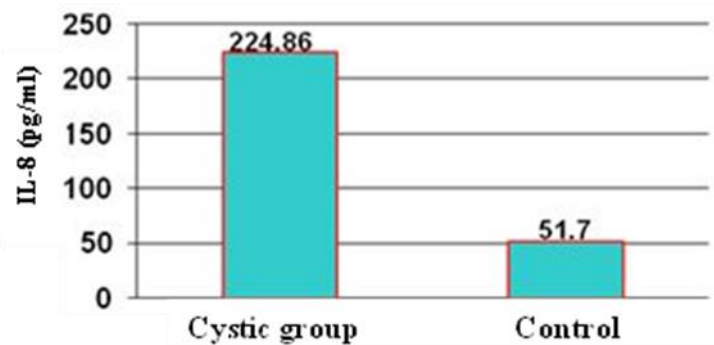


Figure-1: Mean level of urine IL-8 in cystitis patients and healthy control

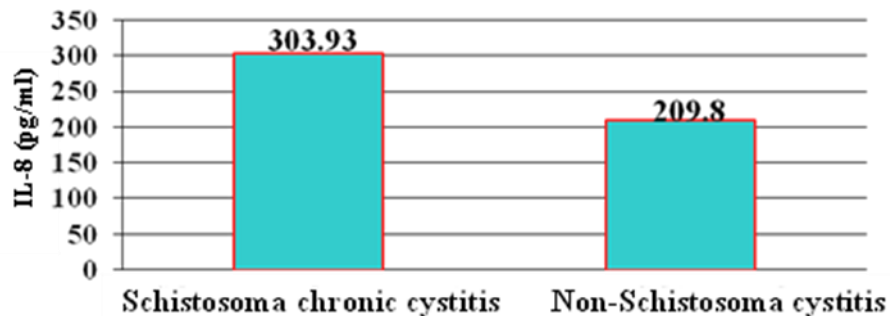


Figure-2: Mean level of IL-8 in Schistosoma chronic cystitis and non-Schistosomal cystitis

**Discussion:**

A cytokine that acts as a chemotactic factor for neutrophils, T-cell subsets and basophils is interleukin-8, it activates neutrophils to undergo a respiratory burst and degranulate in addition to release lysosomal enzymes [11]. IL-8 production is in response to IL-1 beta and TN factor- $\alpha$  [12].

In this recent study it has been noted a highly significant increase (P=0.0001) in IL-8 level in cystitis patients (224.86 pg/ml) when compared with healthy individual (51.7 pg/ml), this was comparable to [11,13].

In considering that urinary IL-8 was significantly more higher in the patients group than healthy, and Rodhe et al., 2009 recorded urinary levels of CXCL8 were

statistically higher in acute cystitis patients than in the asymptomatic bacteriuria (ASB) group<sup>[14]</sup>.

In regard to age and gender IL-8 level showed significant difference ( $P < 0.05$ ) between age groups, the highest level was 272.42 pg/ml in 60-69 age groups and lowest level was 195.05pg/ml in the 40-49 age group, but according to sex IL-8 level was with non-significant difference, this result in agreement with Valavi et al., 2011 who noted no correlation between sex and urine IL-8<sup>[15]</sup>.

However, Taha et al., 2003 noted that IL-8 had been correlated with sex and age<sup>[16]</sup>. While Benson et al., 1996 showed no correlation between urine IL-8 level with the gender and age of the patients with UTI. The different in results of all these mention studies indicate a need for an expanded and more studies in this side with a large sample size.

In Schistosoma chronic cystitis urine concentration of IL-8 (303.93 pg/ml) was increased (significantly) in comparison to that of non- schistosomal cystitis (209.80 pg/ml), IL-8 were positively associated with schistosomiasis intensity<sup>[18]</sup>.

In this study, the urine level of IL-8 was with high sensitivity and specificity (96.34%, 91.39%) when compared with healthy group which indicate that significant increase in cytokines as an early signs for differentiation between healthy and cystitis patients there was an agreement with recent study done by<sup>[15]</sup>. Of whom they found the sensitivities and specificities for CXCL8 to discriminate acute cystitis than ASB were 63% and 96% respectively.

### **Conclusion:**

Significantly high urinary IL-8 levels in patients with cystitis group. With significant increase according to schistosomal infection and age.

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